Doctoral Thesis

Organic matter as source of and sink for photoproduced reactive intermediates

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Organic matter as source of and sink for photoproduced reactive intermediates

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Summary

Natural organic matter (NOM) is an unknown mixture of organic molecules with varying size and composition, which associate to form supramolecular assemblies through hydrophobic interactions and hydrogen bonding. NOM is ubiquitous in the aquatic environment and is known to be involved in numerous environmental processes (e.g., carbon cycling and pollutant fate). One important aspect of NOM relates to its photochemistry in sunlit surface waters. In natural waters, NOM can act as a substrate for direct photoreactions, but also as a sensitizer for the formation of photochemically produced reactive intermediates (PPRI) and quencher of the PPRI. The central aim of this thesis was to gain insights into the structure and reactivity of NOM by studying its role as both a source of and sink for PPRI (e.g., singlet oxygen and hydroxyl radical (HO•)). This thesis covers three aspects of the interaction of NOM and PPRI including the (1) development and characterization of probe molecules for the study of ¹O₂ distribution in NOM-containing aqueous solutions, (2) study of the relative importance of the particulate (POM) and the dissolved (DOM) fraction of NOM in the photochemical production of ¹O₂, and (3) in-depth analysis of the use of HO• quenching by DOM kinetics to assess the number average molecular weight of DOM.

In the first objective, a set of ¹O₂ probe molecules was developed in order to study the formation of ¹O₂ in natural systems. The first goal was to assess the reactivity of furfuryl alcohol (FFA), a well-behaved probe for measuring ¹O₂, under environmentally relevant conditions and the second goal was to modify the FFA structure with various moieties that could partition into NOM assemblies. The kinetic mechanism of FFA reaction with ¹O₂ was shown to be weakly affected by pH, temperature and solvent (i.e., H₂O or D₂O), suggesting that FFA was an ideal
candidate for environmentally relevant studies. In addition, the results suggest that slight modifications to the chemical structure of FFA may be useful to obtain (micro)environment-specific probe molecules. For example, by synthesizing FFA-analogues with varying hydrophobic moieties, partitioning of probe molecules to DOM was explored. Furthermore, modifications to FFA did not affect its reactivity with $^1$O$_2$ and the different hydrophobicity of the probes tested was directly correlated with the concentration of $^1$O$_2$ measured, re-confirming the microheterogeneous distribution of $^1$O$_2$ in NOM containing solutions.

In order to study the relative importance between POM and DOM in the photochemical sensitization of $^1$O$_2$, the reactivity of two probe molecules (i.e., water-soluble and hydrophobic probes) were compared. These studies were essential to the investigation of the average concentration of $^1$O$_2$ generated in a photoirradiated solution containing DOM or POM compared to the $^1$O$_2$ concentration inside the NOM assembly. Photolysis studies were conducted for both solutions containing DOM, as well as either synthetic aggregates of DOM (herein referred to as POM) or natural sediment from a lake as the $^1$O$_2$ sensitizers. The results of these studies indicate that POM is a photosensitizer for $^1$O$_2$, but its overall contribution to the $^1$O$_2$-mediated transformation is limited to reactions in the near proximity of POM.

Finally, the work in this thesis focused on establishing a correlation between the kinetics of HO• quenching by DOM and the molecular weight or size of DOM. This indirect approach was novel in that it was based on the low selectivity of HO• and the consequent assumption of HO• reacting at the same rate with nearly all organic molecules. The molecular weights obtained with this indirect method were lower than literature molecular weights values estimated by conventional analytical techniques. While this study of HO• quenching by DOM does not allow for an
accurate determination of the molecular weight of DOM, the presented approach led instead to new insights into the reactivity and structural properties of DOM.

Overall, the research presented in this thesis contributes to a better understanding of NOM and PPRI interactions and has improved our understanding of the reactivity and structure of NOM.

Sommario

Il materiale organico naturale (NOM) è una combinazione di molecole organiche indefinite di diversa dimensione e composizione, associate fra loro attraverso legami ad idrogeno e interazioni idrofobiche a formare una struttura supramolecolare. 1 NOM è presente negli ambienti acquatici ed è noto per essere coinvolto in numerosi processi ambientali (per esempio nel ciclo del carbonio ² e nella trasformazione di inquinanti ³, ⁴). Un aspetto importante del NOM è la sua partecipazione nella fotochimica delle acque superficiali irraggate dal sole. ⁵ Nelle acque naturali, NOM si può comportare come substrato per fotoreazioni dirette, ma anche come sensibilizzatore per la formazione di intermedi reattivi prodotti fotochimicamente (PPRI) e come quencher di PPRI. Lo scopo principale di questa tesi è di acquisire maggiori informazioni sulla struttura e la reattività del NOM, studiando il suo ruolo di fonte e quencher di PPRI (ad esempio ossigeno singoletto (¹⁰O₂) e radicale ossidrile (HO•)). Questa tesi copre tre aspetti dell’interazione di NOM e PPRI che includono (1) lo sviluppo e la caratterizzazione di molecole sonda per lo studio della distribuzione dell’¹⁰O₂ in soluzioni acquose contenenti NOM, (2) lo studio dell’importanza relativa del particolato (POM) e della frazione dissolta (DOM) di NOM nella produzione di ¹⁰O₂, e (3) un’analisi approfondita dell’uso del quenching del HO• tramite cinetiche del DOM per stabilire il peso molecolare medio del DOM.

Nella prima parte è stato sviluppato un set di molecole sonda per studiare la formazione di ¹⁰O₂ nei sistemi naturali. Il primo obiettivo era di stabilire la reattività dell’ alcool furfurilico (FFA), una molecola che presenta ottime caratteristiche come sonda per l’¹⁰O₂, in condizioni ambientali, mentre il secondo obiettivo era di modificare la struttura del FFA con vari sostituenti che potessero partizionarsi negli aggregati del NOM.
Il meccanismo cinetico della reazione del FFA con l’¹¹O₂ è debolmente dipendente da pH, temperatura e solvente (i.e., H₂O o D₂O), suggerendo che il FFA è un candidato ideale per studi di interesse ambientale. Inoltre il risultato suggerisce che piccole modifiche alla struttura chimica del FFA potrebbero essere utili per ottenere molecole sonda specifiche per il (micro)ambiente circostante.

Ad esempio, è stata studiata la diversa partizione delle sonde molecolari nel DOM sintetizzando analoghi al FFA con frammenti di diversa idrofobicità. È stato osservato che le modifiche al FFA non ne alterano la reattività con l’¹¹O₂ e la diversa idrofobicità delle molecole sonda è stata correlata con la concentrazione dell¹¹O₂, riconfermando la distribuzione microeterogenea del ¹O₂ nelle soluzioni contenenti NOM.

La reattività di due molecole sonda (i.e., solubili in acqua e idrofobiche) è stata paragonata per studiare l’importanza relativa di POM e DOM nella fotoproduzione di ¹O₂. Questo approccio è stato essenziale per studiare la concentrazione media di ¹O₂ generata per irraggiamento di una soluzione contenente DOM e POM e paragonarla alla concentrazione di ¹O₂ all’interno degli aggregati di NOM. Studi di fotolisi sono stati effettuati utilizzando come sensibilizzatore di ¹O₂ sia soluzioni contenenti DOM, sia sospensioni di aggregati sintetici di DOM (chiamati in questo caso POM) o sedimenti naturali di un lago. I risultati di questi studi indicano che il POM è un fotosensibilizzatore per l¹¹O₂, ma il suo contributo per le trasformazioni mediate da ¹O₂ è limitato alle reazioni che avvengono in prossimità del POM.

Infine, in questa tesi è stata studiata la correlazione tra la cinetica del quenching del HO• da parte del DOM e il peso molecolare o dimensione del DOM. Questo approccio indiretto è originale, dato che è basato sulla bassa selettività del
HO• e sulla supposizione che il HO• reagisca alla stessa velocità con tutte le molecole organiche. I pesi molecolari ottenuti con questo nuovo metodo indiretto sono più bassi dei pesi molecolari riportati in letteratura, ottenuti da tecniche analitiche standard. Malgrado questo studio non permetta un’accurata determinazione del peso molecolare del DOM, offre nuove prospettive sulle proprietà strutturali e sulla reattività del DOM.

In generale la ricerca presentata in questa tesi contribuisce a una migliore comprensione delle interazioni di NOM con i PPRI e ha migliorato la nostra comprensione della reattività e della struttura del NOM.

Chapter 1

Introduction
1.1 Introduction

Natural organic matter in aqueous systems

Natural organic matter (NOM) is a complex heterogeneous mixture of organic molecules of varying size and composition, derived from the degradation of plants, animals and microbes\(^1\) as well as anthropogenic inputs.\(^2\) NOM is ubiquitously distributed in aquatic, terrestrial, and atmospheric environments such as oceans, lakes, rivers, soils, sediments, aerosols, and rainwater.\(^3,4\) The composition of NOM is highly variable and analysis with high-resolution mass spectrometry\(^5,6\) and NMR techniques\(^7\) suggests that it contains thousands of compounds. Because of its wide distribution and heterogeneous nature, NOM is involved in numerous environmental processes such as global carbon cycling\(^8\), nutrient bioavailability, and fate and transport of pollutants.\(^9\)-\(^22\)

To understand environmental processes involving NOM, a detailed investigation of its structure and reactivity is essential but the complexity and heterogeneous nature of NOM have challenged such investigations.\(^23,24\) Currently, the molecular structure of NOM is assumed to consist of numerous low molecular weight molecules combined, associated, and stabilized through hydrophobic interactions and hydrogen bonding.\(^25\) NOM is known to be involved in pH buffering,\(^26\) electron donating and accepting reactions,\(^27\) sorption processes,\(^28\) enhancement of the solubility of hydrophobic compounds,\(^29,30\) and photochemical reactions.\(^31,32\) These properties emphasize the role of NOM to facilitate pollutant degradation reactions. However an understanding on a molecular level of the role of NOM in each of these processes is still under debate.

In this thesis, an investigation into the involvement of NOM in photochemical reactions was conducted. The sections below provide an introduction to the photochemistry of NOM, the role of particulate and dissolved organic matter, and
singlet oxygen and hydroxyl radical as relevant reactive intermediates. The following chapters of this thesis focus on NOM as a source of and sink for singlet oxygen (chapter 2 and 3) and as a sink for hydroxyl radicals (chapter 4).

**Photochemistry of natural organic matter**

Natural surface waters are exposed to a substantial photon flux estimated as 1300 kW/m² of sunlight within a year, which corresponds to about $2.6 \times 10^4$ mol of photons/m². A large portion of these photons is absorbed by the chromophoric fraction of NOM in aquatic systems, which can subsequently lead to various reactive processes. The photochemical behavior of NOM is complex because NOM is a mixture of numerous chromophoric organic molecules, which possess different photochemical properties.

In general, environmental photochemistry can occur following two distinct pathways, direct photochemistry and indirect photochemistry. Direct photochemistry refers to the process in which the substrate absorbs light and is transformed subsequently. Indirect photochemistry on the other hand, encompasses the processes in which a sensitizer absorbs light and either the energetically excited sensitizer itself or a subsequently produced reactive intermediate reacts with the substrate. In natural waters, NOM acts as substrate, sensitizer and quencher. Thus, direct photochemical reactions of NOM can cause transformations of NOM known as photo-bleaching. In addition, NOM can take part in indirect photochemical reactions by initiating the formation of reactive intermediates, acting as a sensitizer, and by quenching these reactive intermediates, acting as a substrate. Because NOM is ubiquitous, it is known to be a major photosensitizer in natural waters and the precursor of photochemically produced reactive intermediates (PPRI) such as singlet oxygen ($^{1}\text{O}_2$), peroxyl radicals (ROO•), hydrogen peroxide (H₂O₂), solvated electron (eₐq⁻), superoxide
triplet excited dissolved OM (\(^3\)DOM)\(^{41}\) and hydroxyl radical (HO•).\(^{42-44}\) While these PPRI are short-lived and present only during illumination and at low concentrations, (with the exception of H\(_2\)O\(_2\) that is able to accumulate in solution and can persist in the dark) the high reactivity substantiates their role in various transformation processes.

**Figure 1.1** Photochemistry of NOM: direct and indirect photochemical reactions including photochemically produced reactive intermediates (PPRI).

The function of NOM as a source of and sink for PPRI, as illustrated in Figure 1.1, plays a key role in various environmental processes and thus has significant consequences for carbon cycling, bioavailability of nutrients, and pollutant degradation. In this thesis, we investigated the role of NOM as source of and sink for \(^1\)O\(_2\) (Chapter 2 and 3), and as a sink for HO• (Chapter 4). The central aim of this thesis was to gain insights into the properties of NOM by studying its reactivity.
Role of the particulate versus dissolved fraction of natural organic matter for photochemical processes in natural waters

The production of PPRI has been intensely studied for the dissolved fraction of the organic matter (DOM), however, these processes are not sufficiently understood for particulate organic matter (POM). The yearly riverine transport of NOM to the ocean is composed of 62.5% DOM and 37.5% POM,\textsuperscript{45} making POM a secondary but important source of carbon in natural waters. Despite POMs importance as carbon source and abundance in natural waters, its role in environmental photochemistry has received little attention thus far. It is known that POM is also photochemically active and that light absorption leads to loss of lignin moieties \textsuperscript{46} as well as production of CO,\textsuperscript{47} CO\textsubscript{2},\textsuperscript{48} and low molecular weight DOM.\textsuperscript{49,50,51,52,53} Further studies suggest, that POM can participate in both photosensitization and quenching of photochemistry, but these processes seem to be limited to substrates sorbed to POM.\textsuperscript{54-57} It has been reported that POM may be involved in the formation of \textsuperscript{3}POM\textsuperscript{58}, but other studies show that no production of \textsuperscript{3}DOM or \textsuperscript{1}O\textsubscript{2} upon irradiation of natural POM occurred, which would otherwise be indicative of excited state precursor generated from POM.\textsuperscript{57,56} If POM plays a role in PPRI photosensitization, especially pollutants or microorganisms adsorbed on POM could react with PPRI at the point of formation. The reactivity of POM adds an important degradation pathway for sorbed species that is often ignored in environmental photochemical studies. The open question of the role of POM in the formation of PPRI motivated our POM study as presented in Chapter 3.
Photochemically produced reactive intermediates: Singlet oxygen and hydroxyl radicals

Among the PPRI formed upon irradiation of NOM, we investigated the reactivity of singlet oxygen and hydroxyl radicals and provide an introduction to their role and specificity in environmental processes below.

**Singlet oxygen.** Singlet oxygen, $^1\text{O}_2$, is the first excited state of ground state molecular oxygen. The ground state electronic configuration of molecular oxygen is relatively rare, because it has two unpaired electrons in its valence shell, and therefore occupies a triplet state ($^3\text{O}_2$). Singlet oxygen is generated by energy transfer to $^3\text{O}_2$ from another molecule, a sensitizer, that has been photo-excited to its triplet state (Scheme 1.1).\(^5\)

![Scheme 1.1](image)

**Scheme 1.1** Energy level diagram for environmental formation of singlet oxygen by energy transfer from a photochemically excited triplet state of a sensitizer, e.g., triplet DOM ($^3\text{DOM}^*$) at a reaction rate constant $k_{\text{sens}}$. The radiative decay pathway and non-radiative (thermal) decay pathway, such as inactivation by the solvent, are also schematized. On the right side of the scheme the electron configuration of the triplet state and of the singlet state are also reported.
The energy required to promote ground state oxygen to $^{1}\text{O}_2$ is relatively low (22.5 kcal/mol)\textsuperscript{32} and many sensitizer, including NOM, possess enough energy to do so. Zepp et al. first demonstrated that $^{1}\text{O}_2$ is photochemically produced in natural waters.\textsuperscript{36} Numerous studies in the last three decades have demonstrated and reinforced the indirect photochemical production of $^{1}\text{O}_2$ by excitation of DOM acting as naturally occurring photosensitizer.\textsuperscript{33,60-64}

Singlet oxygen has specific patterns of reactivity that are unique among PPRI.\textsuperscript{61,65} Singlet oxygen can react with olefins following three different mechanisms yielding specific products: [4+2] cycloaddition forming endoperoxide, [2+2] cycloaddition forming dioxetane, or ene-reaction forming allylic hydroperoxide. In addition, $^{1}\text{O}_2$ is known to oxidize phenols and sulfur-containing organic compound. These five characteristic reaction mechanisms are depicted in Scheme 1.2.
Scheme 1.2 Singlet oxygen reaction mechanisms including [4+2] cycloaddition (eq. 1), [2+2] cycloaddition (eq. 2), ene-reaction (eq. 3), phenol oxidation (eq. 4) and sulfide oxidation (eq. 5).

The ability of singlet oxygen to take part in these transformation reactions depends on its lifetime and reactivity with organic molecules. The lifetime of $^{1}$O$_{2}$ in natural waters is shorter than 4 µs.$^{34, 36, 66, 67}$ This short lifetime is a result of deactivation by water, a fast process ($k_{\text{solv}} = 2.5 \times 10^5$ s$^{-1}$)$^{68}$ representing the major mode of relaxation of $^{1}$O$_{2}$. Furthermore, compounds in solution (such as amines or DOM) also deactivate $^{1}$O$_{2}$. Because of its short lifetime, $^{1}$O$_{2}$ does not diffuse very far from the place where it is generated, creating a gradient of $^{1}$O$_{2}$ from its source (e.g.,
DOM) to the bulk of the solution. Assuming $^{1}\text{O}_2$ is produced inside the DOM and given the high rate constant for deactivation by water ($2.5 \times 10^5 \text{ s}^{-1}$),\textsuperscript{68} we can estimate that 99% of the $^{1}\text{O}_2$ is unable to diffuse further than 250 nm from the DOM where it is formed. Thus, one expects a certain concentration of $^{1}\text{O}_2$ inside and within a radius of 250 nm around the DOM and no $^{1}\text{O}_2$ at a distance larger than 250 nm away from the DOM. This model of micro-heterogeneous distribution of $^{1}\text{O}_2$ in solution has been proposed by Latch et al.\textsuperscript{62} and further confirmed by Grandbois et al.\textsuperscript{69} These studies demonstrated such a distribution by comparing the concentration of $^{1}\text{O}_2$ detected in solution and in proximity to the DOM. In order to verify the microheterogeneity of $^{1}\text{O}_2$, Latch et al.\textsuperscript{62} and Grandbois et al.\textsuperscript{69} employed two probe molecules, one that is distributed evenly in the solution, and another one that is selectively concentrated into DOM. The decay rates of these probes were then related to $^{1}\text{O}_2$ concentrations in the microenvironments into which the probes partitioned (i.e., bulk of the solution versus DOM). The approach of using probe molecules in the detection of $^{1}\text{O}_2$ is widespread and preferred to the direct measurement of $^{1}\text{O}_2$ in natural systems, because the intensity of the $^{1}\text{O}_2$ emission is too weak to be useful at low $^{1}\text{O}_2$ concentrations.\textsuperscript{70}

This trend of using probe molecules to study $^{1}\text{O}_2$ resulted in a large number of investigations intended to outline the requirements needed for a good probe molecule, and the development and use of molecules able to (ideally) meet all the requirements. Traditionally the criteria for a good $^{1}\text{O}_2$ probe molecule in environmental studies have been defined as: (1) no direct photolysis or self-sensitization (no absorbance above 290 nm), (2) no radical-initiated autooxidation or polymerization, (3) no interference by its own photooxygenation products (4) no quenching of sensitizer triplets, (5) no or limited and known physical quenching of $^{1}\text{O}_2$, (6) high reaction rate constant with $^{1}\text{O}_2$, (7) no or known dependence of the reaction rate constant with pH, (8) high water
solubility. Haag and coworkers used these criteria to choose furfuryl alcohol (FFA, scheme 1.2, eq. 1) as the preferred $^{1}\text{O}_2$–probe molecule.

To date, FFA is still the most frequently used $^{1}\text{O}_2$–probe molecule among environmental scientists, being still the only commercially available molecule that is known to meet all the above requirements. However, the above-listed criteria are incomplete considering the micro-heterogeneous distribution of $^{1}\text{O}_2$ in solution when photogenerated by DOM. In fact, those criteria are meant to define the ideal probe molecule to detect the average $^{1}\text{O}_2$ steady-state concentration in the bulk of the solution. This average $^{1}\text{O}_2$ steady-state concentration in solution can be misleading because it does not take into account the localized high $^{1}\text{O}_2$ concentration inside and 250 nm around the sensitizer. The lack of selectivity towards microenvironments may underestimate transformation processes by reaction with $^{1}\text{O}_2$ in NOM-bound systems.

The potential underestimation of the exposure to $^{1}\text{O}_2$ of NOM-bound systems, suggests the use of a second probe specific for NOM microenvironments. To date, the state-of-the art approach to fully characterize $^{1}\text{O}_2$ concentrations in natural water is the use of the two probes system introduced by Latch et al.\textsuperscript{62} and Grandbois et al., being FFA the probe for the average $^{1}\text{O}_2$ concentration in solution and a vinyl ether probe, \[2-[(3-(\text{tert-butyldimethylsiloxy})\text{phenyl})\text{methoxymethylene}]\text{adamantane},\] TPMA (Scheme 1.2, eq. 2), the probe specific for NOM microenvironments. However, TPMA is not commercially available and its synthesis is challenging, making it a much less convenient probe for routine analysis. The challenges associated with TPMA motivated our research to investigate a more accessible probe molecule to characterize the $^{1}\text{O}_2$ concentration inside and in proximity to NOM as presented in Chapter 2.
**Hydroxyl radical.** Hydroxyl radical (HO•) is one of the most reactive PPRI and the strongest oxidant in the PPRI family.\(^{31,37}\) The reason for the especially high reactivity is the large dissociation energy of the O–H bond in the water molecule (119 kcal/mol)\(^{72}\) as compared to that of a strong C–H bond, e.g. of CH\(_4\) (105 kcal/mol).\(^{73}\) Thus, HO• is highly unstable and reacts almost indiscriminately with any molecule it encounters. Despite the fact that the high reactivity and the relatively low formation rate of HO• leads to low steady-state concentrations in natural waters (10\(^{-16}\) M), it remains a highly potent oxidant for environmental processes.\(^{37,74}\)

Hydroxyl radicals can be produced in natural waters by four distinct photochemical production pathways: (1) photo-Fenton chemistry, (2) photolysis of nitrate or nitrite, (3) photolysis of H\(_2\)O\(_2\), and (4) photolysis of DOM (Scheme 1.3).\(^{42,43,44,75,76}\)

\[\text{1. } \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{HO•} + \text{OH}^- + \text{Fe}^{3+}\]
\[\text{2a. } \text{NO}_3 \xrightarrow{h\nu} \text{NO}_2 + \text{O}^- + \text{H}^+ \rightarrow \text{HO•}\]
\[\text{2b. } \text{NO}_2 \xrightarrow{h\nu} \text{NO} + \text{O}^- + \text{H}^+ \rightarrow \text{HO•}\]
\[\text{3. } \text{H}_2\text{O}_2 \xrightarrow{h\nu} 2\text{ HO•}\]
\[\text{4. } \text{DOM} \xrightarrow{h\nu} \text{HO•}\]

**Scheme 1.3** Photochemical production pathways of hydroxyl radical (HO•) including photo-Fenton reaction (eq. 1), nitrate and nitrite photolysis (eq. 2a and 2b), H\(_2\)O\(_2\) photolysis (eq. 3) and DOM photolysis (eq. 4).

While the first three processes involve well-defined species, DOM is relatively undefined and system-dependent.\(^{77}\) Furthermore, previous studies suggest that HO• may be formed under non-photochemical conditions through DOM redox pathways.\(^{78,79}\)
Hydroxyl radical reacts following four specific reaction mechanisms: (1) hydrogen atom abstraction, (2) addition to unsaturated bonds, (3) electron transfer, and (4) radical recombination. (Scheme 1.4). The first two mechanisms involve structure that are most commonly observed in organic substrates.

1. \( \text{HO}^\cdot + \text{R-H} \rightarrow \text{H}_2\text{O} + \text{R}^\cdot \\
2. \text{HO}^\cdot + \text{C} = \text{C} \text{ + OH}^\cdot \rightarrow \text{H}^\cdot + \text{HO}^\cdot \\
3. \text{HO}^\cdot + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{Fe}^{3+} \\
4. \text{HO}^\cdot + \text{HO}^\cdot \rightarrow \text{H}_2\text{O}_2

Scheme 1.4. Hydroxyl radical reaction mechanisms including hydrogen abstraction (eq.1), addition to double bonds (eq. 2), electron transfer (eq. 3), and radical recombination reaction (eq. 4)

Because of its strong oxidizing capacity, \( \text{HO}^\cdot \) is able to oxidize otherwise fairly stable molecules such as bisphenol A,\(^{14,21}\) polychlorinated biphenyls (PCBs),\(^80\) and polycyclic aromatic hydrocarbons (PAHs),\(^74\) raising \( \text{HO}^\cdot \) to one of the most relevant reactive oxygen species (ROS) involved in pollutant degradation. Hydroxyl radicals react with most organic molecules at near-diffusion-control rates. Its high reactivity and low selectivity also make \( \text{HO}^\cdot \) one of the primer PPRIs responsible for NOM indirect photobleaching.\(^{33,75,81}\) These observed effects of \( \text{HO}^\cdot \) to alter NOM motivated our study on assessing structural information of NOM based on the \( \text{HO}^\cdot \) quenching kinetics as reported in Chapter 4.
1.2 Goals and overview of the presented work

The aim of this thesis was to investigate different aspects of NOM photochemistry to elucidate advanced information on the reactivity and structural properties of NOM. Therefore, the studies reported in this thesis focused on the interaction of NOM with two PPRI: singlet oxygen (\(^1\)O\(_2\)) and hydroxyl radical (HO•).

In Chapter 2, the reactivity of one commonly used \(^1\)O\(_2\) probe molecule, furfuryl alcohol (FFA), was investigated under environmentally relevant conditions. We further developed a suite of more hydrophobic probe molecules, derived from FFA, which can complement the use of FFA, to investigate the micro-heterogeneous distribution of \(^1\)O\(_2\) in proximity to its source, i.e., NOM. Probe molecules with increasing hydrophobicity were developed with the goal of tuning the partitioning into NOM. With this series of probe molecules, we investigated their application to detect \(^1\)O\(_2\) steady-state concentrations ([\(^1\)O\(_2\)]\(_s\)) in aqueous systems containing NOM.

In Chapter 3, the properties of NOM as a sensitizer for \(^1\)O\(_2\) production were more deeply investigated, specifically focusing on the relative importance of the particulate organic matter (POM) and dissolved organic matter (DOM) fractions. Synthetic POM was prepared by coating silica particles with commercial organic matter (humic acids). The photochemical behavior of this POM was compared to DOM of the same organic matter source used for the coating. The [\(^1\)O\(_2\)]\(_s\) was investigated using two probe molecules: FFA, to establish the average [\(^1\)O\(_2\)]\(_s\) in the bulk of the solution, and TPMA, to establish the [\(^1\)O\(_2\)]\(_s\) inside or in proximity to NOM. This dual approach allowed the assessment of the role of POM for \(^1\)O\(_2\)-mediated degradation of particle-bound pollutants for the first time. In this chapter, we further discussed, by means of a kinetic model, how POM and DOM systems differ regarding the competition of \(^1\)O\(_2\) quenching by NOM and diffusive loss of \(^1\)O\(_2\).
In Chapter 4, the possibility of assessing the average size of DOM with an indirect method based on the reaction kinetics of DOM reaction with HO• was investigated. HO• is widely described as an unselective reactant toward organic molecules. To quantitatively assess this unselectivity, literature values of reaction rate constants of HO• with various organic molecules were surveyed and this allowed the determination of a representative quenching rate constant of HO• with organic molecules. This value, combined with literature HO• quenching constants of humic and fulvic acids, serving as model DOM, were used to estimate the average molecular weight of the various humic and fulvic acids. We compared the molecular weight estimated by our indirect method with molecular weights previously estimated by standard analytical techniques such as size exclusion chromatography, vapor pressure osmometry and flow field fractionation. The discrepancies between our results and previously published results and their implications with regard to the specificity of the reactivity of DOM with HO• are discussed.
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Chapter 2

Effect of pH, temperature and hydrophobicity on the reaction of singlet oxygen with furfuryl alcohol and furfuryl esters

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2.1 Abstract

Furfuryl alcohol (FFA) is the most widely used probe molecule for the detection of singlet oxygen (\(^1\)O\(_2\)) in environmental systems at different pH and temperature conditions. The rate constant for the reaction of FFA with \(^1\)O\(_2\) (\(k_{rxn}\)) has been measured numerous times with different techniques and the reported rate constants cover a relatively wide range (8.3-15 \(10^7\) M\(^{-1}\) s\(^{-1}\)), that could result in up to 45% difference in the measured singlet oxygen concentration, depending on the rate constant used. In the current contribution, a systematic study of \(k_{rxn}\) under different pH and temperature conditions was assessed. A \(k_{rxn}\) value of 1.0 x \(10^8\) M\(^{-1}\) s\(^{-1}\) was determined and no pH dependence in the range of 4 to 10. In addition, only a weak temperature dependence was observed corresponding to a 3-fold increase in the rate constant over a 40 °C range. From the temperature dependence, activation parameters were obtained: \(E_a = 7.4\) kJ mol\(^{-1}\), \(\Delta H^\ddagger = 14.6\) kJ mol\(^{-1}\), \(\Delta S^\ddagger = -44\) J mol\(^{-1}\) K\(^{-1}\). It was further found that the \(k_{rxn}\) value was similar in D\(_2\)O and in H\(_2\)O, showing no evident solvent isotopic effect. The similar reactivity under a wide variety of conditions makes FFA a versatile probe molecule for environmental studies, and suggests that FFA analogues could be useful probes tuned to perform according to the specific microenvironments. As an example, investigation on a series of furfuryl esters with increasing hydrophobic character was performed. The choice of increasing hydrophobicity was aimed at increasing the binding to DOM, ultimately to give a DOM-bound probe with a similar reactivity as FFA. The FFA-based probes were found to share a fast reactivity with \(^1\)O\(_2\) and it was confirmed that the hydrophobic probe molecules detect higher \(^1\)O\(_2\) concentrations. The direct relationship between the hydrophobicity of the probe and the apparent \(^1\)O\(_2\) concentration was taken as a further demonstration that DOM-bound molecules experience enhance concentrations of \(^1\)O\(_2\).
2.2 Introduction

Singlet oxygen ($^1\text{O}_2$) is the first electronic excited state of molecular oxygen and is generated in solution by energy transfer from excited triplet sensitizers. Singlet oxygen is implicated in the photochemical damage of DNA$^1$, proteins$^2$ and cell membranes,$^3$ it has also been shown to be important in the photochemical degradation of certain environmental contaminant classes such as phenols, furans, olefins and sulfides.$^4, 5$ In natural waters, where supramolecularly organized natural organic matter is the sensitizer, $^1\text{O}_2$ exhibits microheterogeneous distribution.$^6, 7$ Elevated concentrations of $^1\text{O}_2$ at NOM compared to the bulk aqueous solution lead to enhanced photooxidation of sorbed contaminants$^8-12$ and NOM itself.

Because $^1\text{O}_2$ is present in biological and environmental systems, there has been a strong interest in understanding its properties and characterizing its distribution in numerous research areas,$^{13}$ spanning from medicine$^{14}$ to biology,$^{15}$ organic chemistry,$^{16}$ physical chemistry and environmental chemistry$^{17}$. Hence, $^1\text{O}_2$ is one of the most broadly studied oxidants. Direct detection of $^1\text{O}_2$ is possible through luminescence emission at 1,268 nm.$^{18}$ However, the intensity of this emission is too weak to be useful at low environmental concentrations ($\sim 10^{-12}$ to $10^{-13}$ M).$^{18}$ This has led to the use of indirect detection methods and the development of numerous $^1\text{O}_2$ trapping probes.$^{18}$ Nardello et al. listed five properties that a probe molecule must meet to be a good $^1\text{O}_2$ probe$^{19}$ and these properties are: (1) high reactivity towards $^1\text{O}_2$, (2) high specificity for $^1\text{O}_2$, (3) compatibility with aqueous media, (4) not perturbing the system, and (5) transparency in the spectral range of incident light to avoid photodegradation and photosensitization by the probe molecule itself.

Meeting these requirements narrows the choice of the wide range of probe molecules reported$^{18}$ to only a handful, including furfuryl alcohol (FFA),
dimethylfuran (DMF) and 1,3-cyclohexadiene-1,4-diethanoate (CHDE). FFA is commercially available (unlike CHDE), selective, sensitive, photostable and water-soluble and has low volatility (unlike DMF) and these properties make it a nearly ideal probe for aqueous \(^1\)O\(_2\) measurements. Consequently, FFA has become one of the most widely used probe molecules in environmental aquatic photochemistry.\(^{18}\)

The reaction rate constant of FFA with \(^1\)O\(_2\) (\(k_{\text{rxn}}\)) has been measured several times using different techniques (Appendix A, Table S1).\(^5,20-23\) The \(k_{\text{rxn}}\) values measured at room temperature in water with different techniques are in the range of 8.3 to \(15 \times 10^7\). These \(k_{\text{rxn}}\) values have been used to determine \([^{1}\text{O}_2]_{ss}\) in solution by dividing the observed decay of FFA (\(k_{\text{obs}}\) in s\(^{-1}\)) by \(k_{\text{rxn}}\).

Two \(k_{\text{rxn}}\) values are commonly used in environmental studies to calculate \([^{1}\text{O}_2]_{ss}\): 1.2 \(\times 10^8\) M\(^{-1}\) s\(^{-1}\) reported by Haag et al. and 8.3 \(\times 10^7\) M\(^{-1}\) s\(^{-1}\) reported by Latch et al.\(^5\) This difference in \(k_{\text{rxn}}\) causes the \([^{1}\text{O}_2]_{ss}\) estimation to differ by 30 percent. The rate constant by Haag et al. (1.2 \(\times 10^8\) M\(^{-1}\) s\(^{-1}\)) was assessed by an indirect method. The authors followed the O\(_2\) depletion electrochemically over time, assuming that the reaction of \(^1\)O\(_2\) with FFA is the only oxygen consuming process. This approach by Haag et al. may result in an overestimation of the rate constant if other oxygen consuming processes were operating. The rate constant reported by Latch et al.\(^5\) (8.3 \(\times 10^7\) M\(^{-1}\) s\(^{-1}\)) was determined from the quenching of \(^1\)O\(_2\) by FFA using laser flash photolysis (LFP). The advantage of the LFP method is that it measures \(^1\)O\(_2\) directly. However, this approach also has the shortcoming that it measures the sum of all quenching pathways including physical quenching or external quenching. It therefore potentially overestimates the \(^1\)O\(_2\) depletion due to reaction with FFA. Another potential weakness of the LFP method is that the measurements were performed using D\(_2\)O as the solvent, and it is unknown whether there is a solvent
isotope effect on the reaction rate constant between FFA and $^{1}$O$_{2}$. The fact that both the Haag and Latch methods for obtaining $k_{\text{rxn}}$ are potentially prone to overestimation, the higher one ($1.2 \times 10^{8}$ M$^{-1}$ s$^{-1}$ by Haag et al.) is arguably more likely to be further from the true value.

Both the Haag and Latch values were determined for a narrow set of solution conditions and in neither case were pH or temperature varied. Environmental studies aimed at assessing $[^1\text{O}_2]_{ss}$ have been performed over a wide range of pH and temperature values, but have used the same $k_{\text{rxn}}$ value for all these conditions.

Early work on the reaction of FFA with $^{1}$O$_{2}$ by Sluyterman indicated no pH dependence between pH 3 and 9 on the rate of O$_2$ consumption in solution as determined by manometry. It would be of interest to extend this range to higher pH values, since many environmental photochemistry studies have been performed at pH 10, for example to study phenolate ions. There is some indication from structurally related probes that the reaction rate constant could be pH dependent above pH 9.

DMF, a furan-based probe similar to FFA, has been shown to have a pH-dependent $k_{\text{rxn}}$ value. DMF’s $k_{\text{rxn}}$ value with $^{1}$O$_{2}$ decreases 50% upon increasing the pH from 3 to 5, has a stable value between pH 5 and 9, and decreasing again upon increasing the pH from 9 to 11.

The temperature dependence of the reaction between FFA and $^{1}$O$_{2}$ is unclear. In organic solvent furans show no temperature dependence ($\Delta H = 0$) in their reaction with $^{1}$O$_{2}$. By contrast, a study by Gottfried et al. reported a 150% increase in the rate constant in the range between 15 and 45 ºC in water. However, other published rate constants in water do not give a consistent picture. For example, Latch measured a rate constant at 30 ºC that was 30% lower than that measured by Haag at 20 ºC. (Appendix A, Table S1)
Probing $^{1}\text{O}_2$ in dissolved organic matter (DOM) solution

FFA is suitable for determining the concentration of $^{1}\text{O}_2$ in the bulk aqueous solution, but to measure the local concentrations of $^{1}\text{O}_2$ experienced by molecules bound to DOM, one must use special probe molecules that bind to DOM. One such probe is the vinyl ether 2-[(1-(3-tert-butyldimethylsiloxy)phenyl)-1-methoxymethylene]adamantane (TPMA).\textsuperscript{6, 7, 25} While the use of TPMA as a probe associating with the DOM has been successful, widespread adoption of TPMA is not expected because it is not commercially available, it is challenging to synthesize, and is prone to decomposition. Moreover the rate constant for reaction of $^{1}\text{O}_2$ with TPMA is slow compared to FFA. As stated by Nardello et al,\textsuperscript{19} fast reacting probe molecules are preferred. In addition, the detection method of TPMA is based on chemiluminescent degradation of the oxygenation product, which requires a precise calibration of the chemiluminometer (CL) with a standard. The standard, which is the dioxetane of TPMA, is also not commercially available and fairly unstable. Finally, comparing results obtained with TPMA and FFA can be challenging because of the strong differences in the two different analytical techniques (i.e., CL for TPMA and liquid chromatography for FFA).

An ideal alternative to TPMA would be a furfuryl-based molecule with a high affinity for DOM. An FFA and FFA-based probe pair may overcome all the above listed concerns with the FFA and TPMA pair.

The work presented here is broken into two parts: First, the reactivity of FFA with $^{1}\text{O}_2$ produced by a homogeneous sensitizer is characterized under different conditions, varying pH and temperature. Second, commercially available or easy to synthesize FFA-based esters (FFRn) are investigated as $^{1}\text{O}_2$ probes (a) with a
homogeneous sensitizer to assess the reactivity with $^{1}\text{O}_2$, and (b) with solutions of DOM of various sources and concentrations to test the effectiveness of the FFRn molecules as DOM-bound $^{1}\text{O}_2$ probes.

### 2.3 Materials and methods

**Chemicals and general methods**

FFA (Merck) was purified by distillation under vacuum, D$_2$O was purchased from Armar, 6-hydroxy-2,3-dihydro-6H-pyrano-3-one (HDP) was purchased from Fluorochem,, and all the other materials were purchased from Sigma-Aldrich at the highest purity grade and used without further purification unless noted. FFA derived probes 3-(furan-2-yl)-1-phenylpropan-1-one (FFRPh), 1-(furan-2-yl)-5,5-dimethylhexan-3-one (FFR5') and 1-(furan-2-yl)tetradecan-3-one (FFR11) ([Figure 1](#)) were synthesized and purified as described in the supporting information. All solvents used for the analysis were of chromatography grade. All aqueous solutions were prepared in ultrapure water (18 M\(\Omega\)·cm, Barnstead Nanopure Diamond system)
**Figure 2.1.** Furan-based singlet oxygen probe molecules. The top panel shows schematically the general reaction of furans with $^{1}O_2$ to form the endoperoxide. The bottom panel summarizes the structures of the employed probes.

**FFA reaction rate constant with singlet oxygen**

In this study we followed a steady-state approach to measure the reaction rate constant of FFA with $^{1}O_2$, $k_{rxn}$. We studied the rate of decay of FFA with time for different initial concentration. Plotting the decay rate versus the initial FFA concentration leads to a saturation-type curve, where, for low FFA concentration, an increase in FFA leads to a linear increase of the rate, whereas for high substrate concentrations, increasing FFA does not affect the rate. The saturation kinetics function is characterized by two parameters, one being the asymptote of the function corresponding to the rate of formation of $^{1}O_2$, and the other one being the half-saturation concentration of FFA, where half of the maximum $^{1}O_2$ formation rate is observed. The rate of decay of FFA over time is expressed the product of the rate constant and the concentrations of $^{1}O_2$ and FFA (Equation 2.1).

$$\frac{d[FFA]}{dt} = k_{rxn}[^{1}O_2]_{ss} [FFA]$$  \hspace{1cm} (2.1)

| FFR1: $R=CH_3$ | FFR4: $R=CH_2CH(CH_3)_2$ |
| FFR2: $R=CH_2CH_3$ | FFR5: $R=CH_2C(CH_3)_3$ |
| FFR3: $R=(CH_2)_2CH_3$ | FFRPh: $R=Ph$ |
| FFR4: $R=(CH_2)_3CH_3$ | FFR11: $R=(CH_2)_{10}CH_3$ |
The singlet oxygen steady state concentration $[^1\text{O}_2]_{ss}$ can be estimated by applying the steady state approximation to the rate of $^1\text{O}_2$ consumption (Equation 2.2-2.3)

$$\frac{d[^1\text{O}_2]_{ss}}{dt} = k_f - k_{solv}[^1\text{O}_2]_{ss} - k_{rxn}[^1\text{O}_2]_{ss} \text{ [FFA]} = 0 \quad (2.2)$$

$$[^1\text{O}_2]_{ss} = \frac{k_f}{(k_{solv} + k_{rxn}[\text{FFA}])} \quad (2.3)$$

where $k_f$ is the rate of formation of $^1\text{O}_2$ and $k_{solv}$ is the rate constant of quenching by the solvent (in our case either $2.5 \times 10^2$ s$^{-1}$ or $1.7 \times 10^4$ s$^{-1}$, for H$_2$O and D$_2$O respectively$^{26}$). Substituting $[^1\text{O}_2]_{ss}$ from Equation 2.3 in Equation 2.1 and rearranging gives Equation 2.4.

$$\frac{d[\text{FFA}]}{dt} = \frac{k_f}{\beta + [\text{FFA}]} \quad (2.4)$$

Where $\beta$ is the half saturation concentration of FFA and is the ratio $k_{solv}/k_{rxn}$.

**Effect of pH and temperature on the reaction rate constant**

We measured the rate of decay of FFA over time with ten different initial FFA concentrations ([FFA]$_0$) as described below. By fitting the $\frac{d[\text{FFA}]}{dt}$ values versus the [FFA]$_0$ we estimated $\beta$ and $k_f$. We performed the experiment in D$_2$O at 26.1°C in aqueous solutions (26.1°C) at different pH values (i.e., pH 4, 5, 6, 8, and 10) and at different temperatures (6.1, 9.6, 14.3, 26.1, 47.2 °C), at pH 8.

Solutions of FFA (10 mL in nanopure water, with 10 mM NaCl) were added to a custom-made cylindrical jacked reactor open from the top. The reactor was connected to a thermostat to keep the temperature constant within 0.1°C during each experiment. A medium pressure mercury lamp with a 365 nm band pass filter was used as light source to irradiate sample solution. Prior to the reaction, the solution was saturated with O$_2$ (Carbagas, O$_2$ 99.999% ) and equilibrated to the desired
temperature and pH by addition of HCl and NaOH. Once the pH and the temperature were stable, the chemical sensitizer (perinaphthenone, PN) was added to a final concentration of 10 µM and the irradiation was started. We choose PN as sensitizer (10 µM, stock solution 10mM in EtOH), because it absorbs in the visible range, has a high \(^1\)O\(_2\) quantum yield (close to 1) and its sensitizing properties can be expected to be independent on the pH.\(^{27}\) Those properties make PN an ideal sensitizer for our set of experiments. During the reaction, the pH was kept constant by addition of KOH through an automatic titrator, and the temperature monitored. Samples were collected every 30 seconds, and the reactions were run for each solution in at least triplicates. The slope of the [FFA] versus time provides the rate of the reaction (d[FFA]/dt, \textbf{Equation 2.3}). Values for \(k_f\) and \(\beta\) were obtained by plotting the rate of the reaction obtained at different initial [FFA] versus [FFA]\(_0\) and fitting the curve to \textbf{Equation 2.3}. We expected the \(k_f\) value to be independent of pH and temperature, if the \(^1\)O\(_2\) quantum yield of PN is pH and temperature independent. The experiments were also assessed in non-pH-adjusted-D\(_2\)O at 26.1 °C.

\textit{Photolysis of FFA-based probes}

The FFA-based probes were distilled under vacuum prior to the reaction. Solutions were placed in a merry-go-round sample holder in borosilicate tubes (10 mL) inside a photo reactor (Rayonet, Southern New England Ultraviolet Co, 4 x 365 nm bulbs). During irradiation, solutions were stirred and ventilation kept the temperature in the reactor constant within 25-30 °C (or say 27.5 ± 2.5). The solutions contained 50 µM FFA and 50 µM FFRn in phosphate buffered nanopure water and 0.05% AcN was added as co-solvent to favor the dissolution of FFRn.
In direct photolysis rate estimation experiments, the solution was irradiated without the addition of sensitizer. In the indirect photolysis rate estimation experiment, 20 μM PN was added to the solutions as $^1\text{O}_2$ photosensitizer. In the test of the FFA-based probes under simulated environmental conditions, three commercially available humic acids (HA) were used as natural DOM-based photosensitizers: Aldrich humic acid (AHA, Sigma-Aldrich), Waskish Peat humic acid (WPHA, standard material purchased from international humic substances society (IHSS)), and Suwannee River Humic Acid (SRHA, standard material purchased from IHSS). The AHA solution was prepared by simple dissolution of the commercially available sodium salt. The WPHA and the SRHA solutions were prepared by dissolving the IHSS materials at pH 10. After complete dissolution of the HA, the pH was adjusted to pH 7. Different concentrations of HA (2-40 mgC/L) were used. The concentration of the HA was assessed by TOC analysis (Shimadzu Corporation, TOC-L analyzer). The screening factor of the different solutions was measured using UV-vis absorbance spectra recorded with a Cary 100 spectrophotometer (Varian).

**Affinity of FFRn probes for HA**

It has been demonstrated that the octanol-water partition coefficient ($K_{OW}$) can be used to give a simple estimation of the partition of an organic compound into HA. SPARC software was used to predict the $K_{OW}$ of the FFRn probe molecules.\(^{28}\)

**2.4 Results and discussion**

In this work, we characterized the reactivity of FFA as a $^1\text{O}_2$ probe under environmentally relevant conditions and we explored possible modifications of FFA
to establish probe molecules with higher hydrophobicity and consequently higher DOM affinity. We confirm that the reactivity of FFA with $^1$O$_2$ is not influenced by the pH in the range investigated (pH 4-10). We further show that reaction rate constant with $^1$O$_2$, $k_{rxn}$, is similar in D$_2$O and in H$_2$O showing that any solvent isotopic effect is not apparent. The measured $k_{rxn}$ that we propose as the correct $k_{rxn}$ to be used for environmental studies with FFA as the $^1$O$_2$ probe is $10.2 \times 10^7$ M$^{-1}$ s$^{-1}$. We further demonstrate that structural modifications of FFA in a FF-esters probes series with increasing K$_{OW}$ show evidence of increasing partition into DOM. With respect to $k_{rxn}$, we demonstrated that the FF-esters probes show slightly slower reactivity with $^1$O$_2$ compared to FFA. With respect to the affinity for DOM, we observed a correlation between increasing K$_{OW}$ and observed [$^1$O$_2$]$_{ss}$ in the FFR$n$ series, however none of the probes reached complete binding, suggesting that a different binding mode, such as electrostatic interaction should be explored in the future.

*Effect of pH on the reaction rate constant*

Figure 2.2 shows the saturation kinetics of the reaction of FFA with $^1$O$_2$ under different pH conditions. Table 2.1 summarizes the results of the experiments including $\beta$ value, $k_f$ and the calculated $k_{rxn}$. Estimated values of $k_{rxn}$ range from 8.3 to 12.2 $10^7$ M$^{-1}$ s$^{-1}$ for the different pH conditions and are in agreement with the rates reported in the literature.$^5,^{20}$ All the experiments were performed at least in triplicates, and the measured error for the different conditions are also reported in Table 2.1. All errors on the estimate of $k_{rxn}$ are within 25%, spanning between 6% and 24%. No correlation with the solution pH and $k_{rxn}$ was observed. Figure 2.2 and Table 2.1 also include the results in D$_2$O, and show no solvent isotopic effect on the reaction under investigation ($k_{rxn} = 1 \times 10^8$ M$^{-1}$ s$^{-1}$). The rate obtained for the reaction in D$_2$O
(9.20±1.53 × 10^7 M⁻¹ s⁻¹) is statistically equivalent to the results in H₂O, confirming that no differentiation between the rates is necessary in studies performed in D₂O and H₂O.

The direct method proposed in this study has the advantage to follow directly the consumption of FFA, compared to the indirect methods proposed by Haag et al.²⁰ and by Latch et al.⁵ The photoreactivity of FFA is well studied and is known not to be significantly susceptible to other degradation pathways under environmental conditions, other than via ¹O₂ oxidation.

Even though FFA is a well-behaved probe molecule, it is generally recommended to follow the product formation and not the consumption of the parent compounds to avoid overestimation of the ¹O₂ concentration due to other probe consumption pathways. To exclude any overestimation of the reaction rate constant due to secondary processes that lead to the consumption of FFA, we further followed the HDP formation. HDP is known to be the major FFA photoprodut (~80% conversion), and its formation has been successfully followed previously.²⁹ The results, however, showed that the kinetics of the hydrolysis from the intermediate endoperoxide to the product HDP is the reaction-limiting step, and it is pH dependent, resulting in poor mass balance with FFA consumption and lack of reproducibility under the experimental conditions. For these reasons, HDP production rates were not used to assess the reaction rate of ¹O₂ with FFA. Even without the product growth confirmation, following only FFA disappearance does not leave much room for overestimation of FFA reactivity. Furfuryl alcohol is not susceptible to direct degradation in contrast to other furan-based probes such as dimethylfuran (DMF) and diphenylfuran (DPF).¹⁸
Figure 2.2. Determination of $\beta$ values by saturation kinetics at 26.1°C and for different pH conditions and in D$_2$O. The fitted parameters for the saturation kinetics correspond to $k_f$ and $\beta$ and are reported with their error as one standard deviation.
Table 2.1. Summary of the reaction rate constant ($k_{rxn}$) measured under different solvent conditions. The $\beta$ values obtained from the fittings and the $^1$O$_2$ rate of formation ($k_i$) are reported.

<table>
<thead>
<tr>
<th>Condition</th>
<th>$k_f$ (µM s$^{-1}$)</th>
<th>$\beta$ (mM)</th>
<th>$k_{rxn}$ (10$^7$ M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4</td>
<td>2.73 ±0.22</td>
<td>2.12±0.84</td>
<td>11.8±2.5</td>
</tr>
<tr>
<td>pH 5</td>
<td>2.93±0.31</td>
<td>2.81±0.61</td>
<td>8.9±1.9</td>
</tr>
<tr>
<td>pH 6</td>
<td>3.00±0.35</td>
<td>3.00±0.72</td>
<td>8.3±2.0</td>
</tr>
<tr>
<td>pH 8</td>
<td>2.31±0.06</td>
<td>2.14±0.13</td>
<td>11.7±0.7</td>
</tr>
<tr>
<td>pH 9</td>
<td>2.96±0.27</td>
<td>3.02±0.56</td>
<td>8.3±1.5</td>
</tr>
<tr>
<td>pH 10</td>
<td>2.89±0.15</td>
<td>2.05±0.26</td>
<td>12.2±1.6</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>-</td>
<td>10.1±4.0</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>2.89±0.05</td>
<td>0.18±0.03</td>
<td>9.2±1.5</td>
</tr>
</tbody>
</table>

Effect of temperature on the reaction rate constant

The data in Figure 2.3 shows the saturation kinetics of the reaction of FFA with $^1$O$_2$ at five different temperatures (6.1 °C, 9.6 °C, 14.3 °C, 26.1 °C, 47.3 °C). We observed a 65% change in the rate constant for a 40° C change in the temperature, and roughly 40% change in the rate constant between temperature 9.6 and 26.1°C. This observation suggests that one should not use the same reaction rate constant at different temperatures.

We used the temperature dependence data to extract the activation parameters for the reaction. Plotting the kinetic data according to the Arrhenius equation, we obtain an activation energy ($E_a$) value of 7.4 ± 1.1 kJ mol$^{-1}$ (Figure 2.4, top panel) and this value is in agreement with reported activation energies of FFA reaction with $^1$O$_2$. The thermodynamic values obtained with Eyring plots give an enthalpy of activation ($\Delta H^\dagger$) of 14.6 ± 2.4 kJ mol$^{-1}$ and an entropy of activation ($\Delta S^\dagger$) of -44.4 ± 8.2 J K$^{-1}$ mol$^{-1}$, also in agreement with reported values for $^1$O$_2$ reactions.
Figure 2.3. Determination of $\beta$ values by saturation kinetics at different temperatures at pH 8. The fitted parameters for the saturation kinetics correspond to $k_r$ and $\beta$ and are reported with their error as one standard deviation.
Figure 2.4. Temperature dependence of FFA reaction with $^1\text{O}_2$. The data are analyzed according to the Arrhenius theory (top panel) and the Eyring theory (bottom panel) to estimate the activation energy and the activation parameters, respectively.

**Effects of structural modifications of the probe molecule on the rate constant with singlet oxygen**

We analyzed $k_{\text{rxn}}$ values for a series of FFRn probes, (Figure 2.1). The value of $k_{\text{rxn}}$ was measured indirectly by comparing the rate of decay of FFA with the rate of
decay of the FFRn under investigation. Starting from the decay rate constant of FFA, and assuming the reaction to be pseudo-first order, the $[^1\text{O}_2]_{ss}$ in solution was quantified. Dividing the observed decay rate constant of the probe under investigation by the $[^1\text{O}_2]_{ss}$ measured with FFA ($[^1\text{O}_2]_{ss}^{\text{FFA}}$, Equation 2.5), the FFRn rate constants were obtained.

$$k_{rxn}^{\text{FFRn}} = \frac{k_{obs}^{\text{FFRn}}}{[^1\text{O}_2]_{ss}^{\text{FFA}}}$$

Table 2.2 summarizes the $k_{rxn}$ values for the FFRn compounds relative to the $k_{rxn}$ value for FFA. We observed that all the FFRn are highly reactive with $^1\text{O}_2$. Esters present slightly lower reactivity than FFA, but the values of $k_{rxn}$ are still in the same order of magnitude. However, the FFRn probes have the disadvantage of slightly degrading also by direct photodegradation under the experimental conditions. We observed that the direct degradation increased with the number of carbon atoms (with the exception of FFR11), but do not have an explanation for this phenomenon. FFR11 has a higher reactivity than FFA, this observation might be caused by the amphiphilic nature of FFR11. The formation of micelles or micelle-like aggregates in solution creates a special environment for FFR11 and affects its kinetics. It is noteworthy that FFR11 kinetics were poorly reproducible and the direct degradation was significantly higher than for the other FFRn tested. Moreover the surfactant nature of the probe causes substantial change of the solution condition and potentially modifies the nature of the DOM in solution. Thus, FFR11 is not a suitable probe for assessing $[^1\text{O}_2]_{ss}$ under environmental conditions.

For each of the FFRn we investigated the solvent isotopic effect on photodegradation to assess whether the apparent direct photochemical reaction of the FFRn might be due to self-sensitized production of $^1\text{O}_2$. Since no enhancement of the direct degradation was observed in D$_2$O, we exclude that FFRn acted as sensitizers.
themselves. This hypothesis was further confirmed by the consistency of FFA degradation kinetics in the presence of different FFRn probes.

Table 2.2. Characterization of FFA esters, FFRn. *

<table>
<thead>
<tr>
<th></th>
<th>$k_{obs,dir}$</th>
<th>$k_{obs,indir}$</th>
<th>$k_{rxn}$</th>
<th>$k_{rxn}/k_{rxn,FFA}$</th>
<th>$\log K_{ow}$ (SPARC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFR1</td>
<td>2.21±0.47</td>
<td>2.12±0.02</td>
<td>6.49±0.02</td>
<td>0.64</td>
<td>1.44</td>
</tr>
<tr>
<td>FFR2</td>
<td>2.40±0.26</td>
<td>2.02±0.17</td>
<td>5.32±0.32</td>
<td>0.52</td>
<td>2.00</td>
</tr>
<tr>
<td>FFR3</td>
<td>4.11±0.85</td>
<td>2.10±0.44</td>
<td>7.15±2.43</td>
<td>0.70</td>
<td>2.52</td>
</tr>
<tr>
<td>FFR4</td>
<td>3.67±0.98</td>
<td>1.94±0.02</td>
<td>5.56±0.08</td>
<td>0.55</td>
<td>3.04</td>
</tr>
<tr>
<td>FFR6</td>
<td>4.61±0.21</td>
<td>1.67±0.16</td>
<td>4.63±0.25</td>
<td>0.45</td>
<td>4.01</td>
</tr>
<tr>
<td>FFR4'</td>
<td>4.87±0.15</td>
<td>1.31±0.01</td>
<td>6.14±0.25</td>
<td>0.60</td>
<td>2.83</td>
</tr>
<tr>
<td>FFRBz</td>
<td>1.21±0.03</td>
<td>1.45±0.04</td>
<td>6.77±0.04</td>
<td>0.66</td>
<td>3.06</td>
</tr>
<tr>
<td>FFR5'</td>
<td>4.87±0.15</td>
<td>1.26±0.02</td>
<td>5.61±0.18</td>
<td>0.55</td>
<td>3.20</td>
</tr>
<tr>
<td>FFR11</td>
<td>12.1±3.8</td>
<td>8.54±1.50</td>
<td>33.20±6.03</td>
<td>3.26</td>
<td>6.27</td>
</tr>
</tbody>
</table>

*The table reports the observed direct rate ($k_{obs,dir}$), the observed indirect rate ($k_{obs,indir}$), the reaction rate constant ($k_{rxn}$), the ratio between FFA and FFRn reaction rate constants ($k_{rxn}/k_{rxn,FFA}$), and the octanol-water partitioning coefficient ($K_{ow}$) measured by SPARC online software.28 All the rates were measured in phosphate buffer (10 mM, pH 7).

Effects of hydrophobicity of the probe molecule on the rate constant and interaction with dissolved organic matter.

We tested the FFRn series in the presence of three different DOM sources (AHA, SRHA, WPHA) and at different DOM concentrations. Data in Figure 2.5 shows that, for all the probe molecules, the $[^1{O}_2]_{ss}$ estimated increases linearly with the concentration of the DOM. The similarity of estimated $[^1{O}_2]_{ss}$ values suggest that the probe molecules have a similar, if any, interaction with DOM. The observation also suggests that the probe molecules considered in this experiment do not associate strongly with the DOM. For completely bound probes, such as TPMA, we expect to reach saturation kinetics, with the shape of a binding isotherm and with an asymptote corresponding to the maximum $[^1{O}_2]_{ss}$ inside the HA.6

Data in Figure 2.5 further shows that the apparent $[^1{O}_2]_{ss}$ detected by different probe molecules increases with their hydrophobicity. Data in Figure 2.6 shows the
enhancement of detected $[^1\text{O}_2]_{ss}$ by the probe molecules relative to the $[^1\text{O}_2]_{ss}$ measured by FFA versus the logarithmic $K_{OW}$ values of the probe molecules in the presence of DOM at 10 mg$_C$ L$^{-1}$. The data show up to five fold higher $[^1\text{O}_2]_{ss}$ for FFR5’ compared to FFA. We notice that the probe molecules bind to AHA more than to SRHA (Figure 2.6), which is in agreement with the results obtained with TPMA by Grandbois et al.$^7$, and previous observation for binding properties of commercially available and standard humic acids.$^{31}$ These observations suggest that the mode of interaction of the FFRn with AHA is similar to the one of TPMA, however, TPMA presented up to 40-fold higher $[^1\text{O}_2]_{ss}$ than FFA indicating that the affinity of TPMA to AHA is significantly higher [See data reported in Chapter 3 of this dissertation].

Our observations confirm that increased hydrophobicity of a molecule increases its degradation by reaction with $^1\text{O}_2$ in the presence of DOM and is in agreement with the theory of microheterogeneous distribution of $^1\text{O}_2$ in DOM solutions. Even though the probe molecules under investigation do not show very high affinity to bind or sorb to DOM, the approach of using modified furans as probe molecules for $^1\text{O}_2$ in hydrophobic environment remains promising. Among the proposed probe molecules, the only one with a high enough log $K_{OW}$ to expect high affinity for DOM is FFR11. However, the experimental challenges associated with FFR11 discussed above, such as micelle formation and surfactant characteristics, outcompete its hydrophobic benefits.

In conclusion, the study of the effect of hydrophobicity of the probe molecules suggests that the modification of FFA to its ester does not undermine its utility as a $^1\text{O}_2$ probe moiety. However, the presented modification approach did not achieve significant interaction with DOM. The use of other hydrophobic groups remains a promising approach to increase hydrophobicity of the probe and association with
DOM. Another approach is the exploration of different binding modes, such as electrostatic interaction with DOM. Such charged probe molecules might be a valid solution to overcome the issues of self-aggregation linked to very hydrophobic probe molecules and eliminate the necessity of co-solvents. Preliminary results and further discussion about hydrophobic and charged probes is reported in Appendix A.

**Figure 2.5.** Apparent steady-state singlet oxygen concentration, $[^1\text{O}_2]_{\text{ss}}$, at different dissolved organic matter concentrations, [HA], for different probe molecules and different DOM sources (Waskish Peat humic acid (WPHA), Suwannee River humic acid (SRHA)).
Figure 2.6. The steady-state singlet oxygen concentration, \([1^1O_2]_{ss}\) detected versus the hydrophobicity of the probe molecules for three different sensitizers: Aldrich humic acid (AHA), Waskish Peat humic acid (WPHA) and Suwannee River humic acid (SRHA).

2.5 Environmental implications

We investigated the properties of different FFA-based probe molecules for the assessment of \([1^1O_2]_{ss}\). First, we reinvestigated the reactivity of FFA under different environmental conditions. We extended the pH range studied for FFA to pH 10 and found that FFA displays no pH dependence in its reactivity with \(^1O_2\). We measured an average \(k_{rxn}\) value of \(1.0 \times 10^8\) M\(^{-1}\) s\(^{-1}\) at 26.1 °C. In addition to having no pH dependence, FFA showed very weak temperature dependence. FFA’s rate constant for reaction with \(^1O_2\) changed only 65% over a 40 °C temperature range. This corresponds to an activation energy for this reaction of \(7.4 \pm 1.1\) kJ mol\(^{-1}\), a \(\Delta H^\ddagger\) of \(14.6 \pm 2.4\) kJ mol\(^{-1}\) and \(\Delta S^\ddagger\) of \(-44.4 \pm 8.2\) J mol\(^{-1}\) K\(^{-1}\). We further verified that the
reactivity of FFA is similar in D₂O and H₂O. Second, we proposed a suite of easily accessible FFRn as potential DOM-binding probe molecules to assess the [¹⁰₂]ss experienced by DOM-bound molecules. We verified that the ester group does not significantly affect the reactivity towards ¹⁰₂ and all the proposed probes have $k_{rxn}$ values of the same order of magnitude as FFA. We investigated the behavior of FFRn in the presence of DOM and verified that increasing the hydrophobicity of the probe molecule caused faster degradation in the presence of DOM, which we attribute to higher association with DOM and thus higher [¹⁰₂]ss. None of the probes under investigation were found to completely partition to DOM, suggesting that more investigation needs to be done in this direction. The data suggest that the approach of using modified FFA-based probes is promising and we recommend further investigation in the direction of different binding modes in the future.
2.6 References

19. Nardello, V.; Azaroual, N.; Cervoise, I.; Vermeersch, G.; Aubry, J.-M., Synthesis and photooxidation of sodium 1, 3-cyclohexadiene-1, 4-diethanoate: A new


### 2.7 Appendix A: Supporting material for Chapter 2

#### Table S2.1. Published rate constants for the reaction of furfuryl alcohol with $^{1}O_2$

<table>
<thead>
<tr>
<th>$k_{\text{rxn}}$ (M$^{-1}$ s$^{-1}$)</th>
<th>T (°C)</th>
<th>pH</th>
<th>Solvent</th>
<th>Sensitizer</th>
<th>Detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 $\times$ 10$^8$</td>
<td>45</td>
<td>7</td>
<td>H$_2$O</td>
<td>TPPS$^a$</td>
<td>O$_2$ consumption electrochemically$^1$</td>
</tr>
<tr>
<td>1.9 $\times$ 10$^8$</td>
<td>35</td>
<td>7</td>
<td>H$_2$O</td>
<td>TPPS$^a$</td>
<td>O$_2$ consumption electrochemically$^1$</td>
</tr>
<tr>
<td>1.4 $\times$ 10$^8$</td>
<td>25</td>
<td>7</td>
<td>H$_2$O</td>
<td>TPPS$^a$</td>
<td>O$_2$ consumption electrochemically$^1$</td>
</tr>
<tr>
<td>9.0 $\times$ 10$^7$</td>
<td>15</td>
<td>7</td>
<td>H$_2$O</td>
<td>TPPS$^a$</td>
<td>O$_2$ consumption electrochemically$^1$</td>
</tr>
<tr>
<td>1.5 $\times$ 10$^8$</td>
<td>37</td>
<td>4-9</td>
<td>H$_2$O</td>
<td>Proflavin</td>
<td>O$_2$ consumption Wartburg apparatus$^2$</td>
</tr>
<tr>
<td>1.2 $\times$ 10$^8$</td>
<td>22</td>
<td>7</td>
<td>H$_2$O</td>
<td>Rose Bengal</td>
<td>O$_2$ consumption electrochemically$^3$</td>
</tr>
<tr>
<td>1.2 $\times$ 10$^8$</td>
<td>19</td>
<td>10-11.5</td>
<td>H$_2$O</td>
<td>Rose Bengal</td>
<td>O$_2$ consumption electrochemically$^4$</td>
</tr>
<tr>
<td>8.3 $\times$ 10$^7$</td>
<td>30</td>
<td>7.1</td>
<td>D$_2$O</td>
<td>Rose Bengal</td>
<td>Laser flash photolysis$^5$</td>
</tr>
</tbody>
</table>

$^a$ Meso-tetraphenylporphyrin tetrasulfonate
**Synthesis of furfuryl esters**

a) 1-((furan-2-yl)tetradececan-3-one (FF-R11)

b) 3-((furan-2-yl)-1-phenylpropan-1-one (FFPh)

c) 1-((furan-2-yl)-5,5-dimethylhexan-3-one (FF-R5′)

**Scheme S2.1:** synthesis of three furfuryl esters a) FF-R11, b) FF-Ph, c) FF-R5′

In a two-necked round bottom flask equipped with an addition funnel, furfuryl alcohol (FFA, 1.470 g, 15 mmol, 1 equiv) was dissolved in dry pyridine (15 mL) under N₂. Dichloromethane (15 mL) was added to the mixture. The mixture was cooled to 0 °C with an ice bath and acyl chloride (3.280 g, 15 mmol of (a), 2.108 mg, mmol of (b) and 2.019 mg, 15 mmol of (c), 1 eq) were added dropwise over 30 min. Upon completion of the addition, the reaction mixture was allowed to warm to room temperature and was stirred overnight. The solution was quenched with a saturated aqueous NaHCO₃ solution (50 mL), then the organic phase was extracted with CH₂Cl₂ (3 × 50 mL), washed once with brine (50 mL) and dried with Na₂SO₄ before the solvent was evaporated under reduced pressure. Purification yielded the desired furfuryl esters (FFRn) as follows:
a) FFR11 Purification: flash chromatography, SiO$_2$, EtOAc:Hexane 1:3, yield 50%. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 0.88 (t, $J$=6.66 Hz, 3H, methyl), 1.25 (s, 16H methylene), 1.62 (tt, $J$=7.4 Hz, 14.8 Hz, 2H, methylene), 2.32 (t, $J$=7.25 Hz, 2H, methylene), 5.06 (s, 2H, methylene), 6.35 (dd, $J$=3.33 Hz, 1.83 Hz, 1H, furan (C3)), 6.39 (d, $J$=3.2 Hz, 1H, furan (C4)), 7.42 (dd, $J$=1.9 Hz, 0.7 Hz, 1H, furan (C5)).

b) FFRBz Purification: flash chromatography, SiO$_2$, EtOAc:n-hexane 1:1, yield 86%. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 5.31 (s, 2H, methylene), 6.39 (dd, $J$=3.20 Hz, 1.92 Hz, 1H, furan(C3)), 6.49 (d, $J$=3.2 Hz, 1H, furan(C4)), 7.43 (t, $J$=7.52 Hz, 2H, Bz(C3,C5)), 7.45 (s, 1H, furan(C5)), 7.54 (tt, $J$=7.41 Hz, 1.32 Hz, 2H, Bz(C4)). 8.04 (dd, $J$=8.39 Hz, 1.48 Hz, 2H, Bz(C2,C6)).

c) FFR5’ Purification: vacuum distillation, 70 °C, yield 65%. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.00 (s, 9H, Me), 2.22 (s, 2H, methylene), 5.05 (s, 2H, methylene), 6.35 (dd, $J$=3.22 Hz, 1.93 Hz, 1H, furan (C3)), 6.39 (d, $J$=3.2 Hz, 1H, furan (C4)), 7.41 (d, $J$=1.6 Hz, 1H, Furan (C5)).
**Calculation of screening factors**

The singlet oxygen steady state concentration ([\(^1\text{O}_2\)\text{ss}]) observed with the different ester probes in the presence of different concentrations of organic matter (OM) was corrected for light screening. The screening factor, \(S\), is described by **Equation S2.1**, where \(\alpha\) is the decadic optical density at each wavelength, \(\lambda\) (nm), and \(z\) (cm) is the optical path length.

\[
S_\lambda = \frac{1 - 10^{\alpha_\lambda z}}{2.303 \alpha_\lambda z}
\]  

**Equation S2.1**

The relative light intensity experienced by OM was estimated as follows,

\[
I = \sum_\lambda S_\lambda \times E_\lambda
\]

**Equation S2.2**

where \(I\) is the relative light intensity experienced by the sample and \(E_\lambda\) is the relative irradiance of the UV light.

The absorption spectra were measured in 1 cm quartz cuvettes using a Cary 100 spectrophotometer (Varian) for all the concentrations of Aldrich humic acid (AHA), Suwanee river humic acid (SRHA) and Waskish peat humic acid (WPHA). The values of \(E_\lambda\) were recorded with a spectrometer (OceanOptics Inc.).

Light screening was calculated for the wavelength range in which chromophoric DOM absorbs light (345-410 nm) and the light source emits light. The estimated \([^1\text{O}_2]_{\text{ss}}\) by the different probe molecules were corrected for light screening by dividing the \([^1\text{O}_2]_{\text{ss}}\) by the estimated \(I\) value. Data in **Table S2.2** summarizes relative light attenuation for each DOM solution.
Table S2.2. Relative light intensity ($I$) and relative light attenuation in percent for organic matter solutions.

<table>
<thead>
<tr>
<th>[HA] (mge/L)</th>
<th>$I$ (AHA) (%)</th>
<th>Attenuation (%)</th>
<th>$I$ (WPHA) (%)</th>
<th>Attenuation (%)</th>
<th>$I$ (SRHA) (%)</th>
<th>Attenuation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>91.7</td>
<td>8.3</td>
<td>92.0</td>
<td>8.0</td>
<td>92.8</td>
<td>7.2</td>
</tr>
<tr>
<td>2.5</td>
<td>88.5</td>
<td>11.5</td>
<td>86.3</td>
<td>13.7</td>
<td>89.2</td>
<td>10.8</td>
</tr>
<tr>
<td>4.5</td>
<td>84.7</td>
<td>15.3</td>
<td>80.8</td>
<td>19.2</td>
<td>82.6</td>
<td>17.4</td>
</tr>
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<td>5.1</td>
<td>77.7</td>
<td>22.3</td>
<td>64.5</td>
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<td>76.6</td>
<td>23.4</td>
</tr>
<tr>
<td>12.1</td>
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<td>68.5</td>
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<td>69.8</td>
<td>30.2</td>
</tr>
<tr>
<td>20.7</td>
<td>53.5</td>
<td>46.5</td>
<td>58.6</td>
<td>41.4</td>
<td>61.6</td>
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<td>50.6</td>
<td>49.4</td>
<td>54.1</td>
<td>45.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preliminary investigation of additional singlet oxygen probe molecules

Hydrophobic interaction: FF-TBS

Synthesis of FF-TBS. To overcome challenges associated with the surfactant nature of linear aliphatic furfuryl based probes and with the need for a more hydrophobic furan based compound that mimics the behavior of TPMA, we synthesized FF-TBS (Schematic S2). The silyl group of FF-TBS confers a highly hydrophobic character.

Synthesis of tert-butyl(furan-2-ylmethoxy)dimethylsilane (FF-TBS)

Scheme S2.2. FF-TBS protection of furfuryl alcohol.

The synthesis procedure was adapted from Kendall et al.\(^6\) Furfuryl alcohol (1.560 g, 16.1 mmol, 1 eq., tert-butyl(dimethyl)silyl chloride (4.879 g, 32.3 mmol, 2 eq.) and imidazole (3.317 mg, 48.3 mmol, 3 eq.) and 4-dimethylaminopyridine (0.201 g, 1.61 mmol, 0.1 eq.) were placed in a round bottom flask and dissolved in a solution of dry, distilled dimethylformamide (DMF) in dry, distilled CH\(_2\)Cl\(_2\) (0.1 M, 200 mL) and stirred at room temperature overnight. The reaction mixture was quenched with saturated NH\(_4\)Cl aqueous solution (50 mL), the product was extracted with CH\(_2\)Cl\(_2\) (3 \(\times\) 50 mL) and washed with brine. The organic phase was dried with MgSO\(_4\) and solvent was removed by rotary evaporation. The product was purified by flash chromatography (SiO\(_2\), 1:9 EtOAc:n-hexane). To monitor the flash chromatography
TLC slides were exposed to iodine, to stain the product otherwise not visible by UV light exposition of the plates. The purified product was isolated in 75% yield $^1H$-NMR (400 MHz, CDCl$_3$) $\delta$: 0.08 (s, 6H MeSi), 0.90 (s, 9H t-Bu) 4.64 (s, 2H, Methylene), 6.23 (dd, $J=2.89$ Hz, 0.58 Hz, 1H, furan (C3)), 6.32 (dd, $J=3.12$ Hz, $J=1.74$ 1H, furan (C4)), 7.38 (q, $J=1.8$ Hz, 0.8 Hz 1H, furan (C5)).

Use of FF-TBS as singlet oxygen probe molecule

The FF-TBS probe was tested with the same procedure as described in the material and method section of the main manuscript for the other probe molecules. The reaction rate constant with $^1$O$_2$ ($k_{rxn}$) for FF-TBS was estimated to be $8.93 \times 10^7$ M$^{-1}$s$^{-1}$, according to the procedure used for the FF-esters, described elsewhere (material and methods section), while negligible direct degradation was observed.

Despite the fact that the hydrophobicity of the FF-TBS probe is greater than the other FFA-based probes (and thus closest to TPMA), we observed unexpected results. The chromatographic peak of FF-TBS measured by HPLC (Waters symmetry C18, 3.5 $\mu$m, 4.6x75 mm column, mobile phase 80% AcN, 20% pH5 ammonium acetate buffer 10 mM) is linear with the concentration, the retention time reproducible and the peak sharp and symmetric. However, in the presence of DOM, FF-TBS detected a lower $[^1$O$_2]_{ss}$ compared to what was estimated with FFR5’ as the probe molecule (Figure S2.1).
Figure S2.1. Apparent \([{}^1\text{O}_2]\)\(_{\text{ss}}\) relative to FFA for probes with different \(K_{\text{OW}}\) in the presence of 10mg/L WPHA. The apparent \([{}^1\text{O}_2]\)\(_{\text{ss}}\) for FFTBS (log \(K_{\text{ow}}\) 5.22) is represented in red and is just 2.5 times higher than FFA (log \(K_{\text{ow}}\) 0.18).

We have two possible explanations for these results: 1. the highly hydrophobic protecting group (tert-butyl-dimethyl-silyl-, TBS) caused the FF-TBS probe to strongly self-aggregate, 2. FF-TBS may react slower in the DOM microenvironment than in water. Regarding hypothesis 1, the overall linear behavior of the probe when using perinaphtenone (PN) as the sensitizer and the linear calibration of FF-TBS by HPLC seem to at first exclude aggregation being the reason for a slower reaction rate with \({}^1\text{O}_2\). Regarding hypothesis 2, it is possible that FF-TBS reacts differently when partitioned into DOM, if we consider DOM behaving as a solvent. If, for one of the above reason, the \(k_{\text{rxn}}\) of FF-TBS was overestimated, the calculated \([{}^1\text{O}_2]\)\(_{\text{ss}}\) would be underestimated. Further investigation is recommended to fully understand the behavior of FF-TBS as a \(^1\text{O}_2\) probe. If instead, the self-aggregation linked to the three-dimensional structure of FF-TBS is the reason for a low affinity to DOM, then
we recommend structural modification in the direction of a more hindered R-group to be attached to the furan probe. In Figure S2.2 we propose some possible modifications to the furan probe molecule.

\[ \text{Figure S2.2.} \text{ Possible furan probes that could overcome the aggregation issue and at the same time partition into DOM. The silyl ether (Si) will increase the hydrophobicity and the hindered group such as tert-butyl or adamantane will prevent aggregation. We expect the tert-butyl group will be hindering enough since the FFR5' ester probe offered the best kinetic results and higher DOM affinity.} \]
**Electrostatic interaction: FF-amines**

An additional class of compounds that we have explored as FFA-based probes with some preliminary tests are FF-amines. We made preliminary studies with one commercially available and one synthetic probe. (Figure S2.3).

![Figure S2.3](image)

**Figure S2.3** FF-amines proposed to investigate the internal concentration of singlet oxygen using a probe that binds to DOM by electrostatic interactions.

**Synthesis of FF-amines.** Compound 1, FFN, was tested but showed no binding to DOM, and compound 2, FFPy, has been synthesized but not difficulties in developing and analytical method for FFPy have prevented testing of the compound as a probe.

Since the hydrophobic interactions were also associated with solubility issues, we propose the idea of using electrostatic interaction as a binding mode to DOM. Binding to DOM through electrostatic interaction is expected to be stronger compared to the binding due to hydrophobic interaction. We first investigated a commercially available probe (Figure S2.2, compound 1, FFN). The FFN probe, has shown excellent probe behavior since it is highly reactive towards $^1O_2$ (see below), specificity for $^1O_2$, compatible with aqueous media, and transparent in the spectral range showing no direct degradation, showing identical results to FFA. No direct photochemical degradation was observed, and the degradation rate constant in the presence of the sensitizer PN was identical to the one observed for FFA at pH 7, when
more than 90% of FFN is protonated (pK<sub>a</sub> of 8.4). The \( k_{\text{rxn}} \) value of FFN is slightly dependent on pH, as summarized in Table S2.3, but the dependence is not very strong, with an increase of the rate from pH 3 to pH 8 of 30%.

**Table S2.3. Characterization of the pH dependence of the reaction rate constant of FFN with singlet oxygen relative to data for FFA**

<table>
<thead>
<tr>
<th>pH</th>
<th>( k_{\text{obs}} ) FFA ((\times 10^{-4} \text{ s}^{-1}))</th>
<th>( k_{\text{obs}} ) FFN ((\times 10^{-4} \text{ s}^{-1}))</th>
<th>( ^1\text{O}_2 ) in solution ((\times 10^{-12} \text{ M}))</th>
<th>( k_{\text{rxn}} ) FFN ((\times 10^8 \text{ M}^{-1} \text{s}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.2</td>
<td>5.7</td>
<td>6.1</td>
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<td>5.6</td>
<td>6.1</td>
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<td>6.4</td>
<td>6.2</td>
<td>1.04</td>
</tr>
<tr>
<td>8</td>
<td>6.1</td>
<td>7.8</td>
<td>6.0</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Unfortunately, the FFN probe has a similar reactivity as FFA in the presence of DOM, giving no indication of binding to DOM, which would result in a higher \( k_{\text{rxn}} \). Droge et al.\(^7\) have observed that aliphatic quaternary amines are the least susceptible to electrostatic interaction with DOM.\(^7\) Their studies suggested that aromatic quaternary amines have the highest affinity to DOM and present the highest binding of cationic amines. The Droge et al study stimulated the idea of synthesizing a furfuryl pyridinium (FF-Py) probe for further investigations (Figure S3).

**Synthesis of 1-(furan-2-ylmethyl)-pyridinium**

![Scheme S2.3](image)

**Scheme S2.3** Synthesis of 1-(furan-2-ylmethyl)-pyridinium (FFPy)
In a two neck round bottom flask equipped with an addition funnel and under dry N₂ atmosphere, methanesulfonyl-chloride (9.6 mL, 40 mmol, 1 eq.) was dissolved in CH₂Cl₂ (150 mL) and the solution was cooled to 0 °C by an ice bath. Furfuryl alcohol (3.6 mL, 42 mmol, 1.05 eq.), was added to the solution and upon completion of the addition, pyridine (9.6 mL, 200 mmol, 5 eq.) was added drop-wise over 1 h. The reaction was slowly allowed to warm up to room temperature and stirred overnight. The reaction mixture was quenched with saturated NH₄Cl aqueous solution (50 mL) and the pH adjusted to 7. The excess of pyridine was removed by extraction with CH₂Cl₂ (2 × 50 mL). The water solvent was removed by rotary evaporation and the product was recrystallized from EtOH, washed with pentane and cold EtOH. The purified product was isolated with a 25 % yield.

\[ ^1H-NMR \ (400 MHz, D_2O) \delta: 5.89 \ (s, 2H, \text{methylene}), 6.58 \ (t, J=2.8 Hz, 1H, \text{furan (C3)}), 6.86 \ (d, J=3.1 Hz, 1H, \text{furan (C4)}), 7.64 \ (s, 1H, \text{furan (C5)}), 8.11 \ (t, J=7.2, 2H, \text{pyridine (C3,C5)}), 8.60 \ (t, J=7.95 Hz, 1H, \text{pyridine(C4)}), 8.92 \ (d, J=6.1 Hz, 2H, \text{pyridine(C3,C6)}) \]

We expect this probe to be promising as completely DOM-bound molecules, to compare with FFA in the study of the microheterogeneous distribution of ^1O₂ in DOM solutions. However we encountered technical difficulties in the analytical detection of the FF-pyridinium probe, relegating this investigation at an early stage.
References

Chapter 3

Photochemical production of singlet oxygen from particulate organic matter

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Submitted to:

Environmental Science & Technology
3.2 Abstract

Dissolved organic matter is established as one of the most relevant photosensitizers in aquatic environments, producing singlet oxygen (\(^{1}\text{O}_2\)) alongside other photochemically produced reactive intermediates. While the production of \(^{1}\text{O}_2\) from DOM has been well studied, the relative importance of particulate organic matter (POM) to the overall \(^{1}\text{O}_2\) production is less well understood. POM is known to play an important role in pollutant fate through the sorption and transport of hydrophobic pollutants. If POM is directly involved in \(^{1}\text{O}_2\) production, sorbed molecules would be expected to undergo enhanced photodegradation. In this work, synthetic POM was prepared by coating silica particles with commercial humic acid. The photochemical behavior of these POM samples was compared to dissolved commercial humic acids (DOM). Suspended natural sediment was also studied to test the environmental relevance of the synthetic POM model. Synthetic POM particles appear to simulate well the \(^{1}\text{O}_2\)-production of suspended sediment. The \(^{1}\text{O}_2\)
concentrations experienced by POM-sorbed probe molecules was up to 30% higher than experienced by DOM-sorbed ones, even though the aqueous concentration of $^1$O$_2$ in irradiated POM suspensions was much lower than the analogous DOM solutions. These results were interpreted with a reaction-diffusion model, which suggested that the production rate of $^1$O$_2$ by POM is lower than DOM, but the loss of $^1$O$_2$ from the POM-phase is also lower than DOM. Based on the experimental results of this study, calculations were conducted to estimate the impact of removing POM on $^1$O$_2$-mediated processes. These calculations indicate that compounds with a log $K_{oc}$ value near 4 will be most affected by removal of POM and that the magnitude of the effect is proportional to the fraction of the total organic matter represented by POM. This study demonstrates that particles can play an important role in the degradation of organic compounds via aquatic photochemistry.

### 3.3 Introduction

The role of particulate organic matter (POM) in environmental photochemistry has received much less attention than that of dissolved organic matter (DOM). It is known that POM is photochemically active, as irradiation of POM leads to loss of lignin marker compounds as well as production of CO, CO$_2$, and DOM. In addition, excited state transients have been observed upon irradiation of POM. There is also evidence that POM can participate in both photosensitization and quenching of photochemistry. This is similar to DOM, but with the important distinction that the reactions involving POM appear to be limited to sorbed species. For example, the presence or absence of particles made little difference in the photooxidation of aqueous probes of singlet oxygen ($^1$O$_2$) and triplet state oxidants, furfuryl alcohol (FFA) and trimethylphenol, respectively.
Indirect photochemical reactions involving organic matter-sensitized production of reactive oxidants are well established for DOM. The absorption of sunlight by the chromophoric fraction of DOM results in the formation of a suite of photochemically produced reactive intermediates (PPRI) such as triplet excited states of DOM ($^3$DOM)$^{10, 15-21}$ superoxide/hydroperoxyl radicals ($-\text{O}_2^{-}/\text{HO}_2^-$)$^{22-25}$ hydroxyl radical (HO$^-$)$^{26-29}$ and $^1\text{O}_2$. The production of PPRI from DOM is quite general,$^{24, 34}$ being observed from DOM obtained from freshwater,$^{35}$ marine$^{35, 36}$ and wastewater$^{37}$ samples. While it is expected that PPRI should also be generated by POM, this has yet to be established.

The photoreactivity of POM-sorbed compounds might be analogous to the photochemistry of DOM-sorbed compounds. Sorption to DOM enhances the photochemical degradation of mirex$^{38, 39}$ and methylmercury.$^{40-42}$ Association also strongly enhances the photooxidation of the hydrophobic $^1\text{O}_2$ probe compound 2-[1-(3-tert-butyldimethylsiloxy)phenyl]-1-methoxymethylene]adamantane (TPMA).$^{32, 43}$ In the case of TPMA oxidation, the enhancement is believed to be due to the high local concentrations of $^1\text{O}_2$.

Among the PPRI formed by irradiation of organic matter, $^1\text{O}_2$ is a compelling one to study. It is known to play an important role in the oxidation of pollutants such as phenols, furans, olefins and sulfides$^{44}$ and is involved in the oxidation of DOM itself.$^{45}$ $^1\text{O}_2$ reacts with proteins,$^{46}$ DNA$^{47}$ and biomolecules and is also known to cause oxidative stress in cells$^{48}$, thus, it is capable of causing oxidative damage to cells and to DNA, inducing cell death and inactivation of pathogens.$^{49, 50}$ Due to its relatively short lifetime in water (4 $\mu$s)$^{32}$, $^1\text{O}_2$ is microheterogeneously distributed in aqueous solution with dilute sensitizers, such as when DOM is employed as a sensitizer. With POM as a $^1\text{O}_2$ sensitizer, the microheterogenous distribution of $^1\text{O}_2$ is
expected to be even more dramatic than with DOM since the organic matter is condensed into larger assemblies, which are predicted to have higher “internal” concentrations based on reaction-diffusion kinetic modeling.\textsuperscript{32, 43}

If POM is active in generating \(^1\)O\(_2\), hydrophobic and positively charged pollutants or microorganisms adsorbed on POM could react with \(^1\)O\(_2\) at the site where it is formed. Thus, POM may play an important role in \(^1\)O\(_2\)-mediated disinfection and pollutant degradation, while remaining unimportant for freely dissolved ones. In environmental studies, particles are commonly removed by filtration prior assessment of photochemical processes and thus POM-associated processes are not accounted for and might result in an underestimation of ROS-mediated transformation reactions.

To investigate the role of POM in \(^1\)O\(_2\) production relative to DOM, we measured the \(^1\)O\(_2\) steady state concentration experienced by a probe molecule sorbed to the OM ([\(^1\)O\(_2\)\textsubscript{OM}]) and in the aqueous phase ([\(^1\)O\(_2\)\textsubscript{aq}]) using two known selective \(^1\)O\(_2\) scavengers as probe molecules: the hydrophobic TPMA to assess [\(^1\)O\(_2\)\textsubscript{OM}] and a water-soluble furan-based probe, FFA, for [\(^1\)O\(_2\)\textsubscript{aq}]. FFA is a widely used \(^1\)O\(_2\) probe molecule that is selective, hydrophilic, and photostable.\textsuperscript{30, 31, 51-53} The detection method is based on the decrease in HPLC absorbance peak area. Because of its commercial availability, high solubility in water and well-known reactivity, FFA is the most widely used \(^1\)O\(_2\) probe molecule for environmental studies.\textsuperscript{51} TPMA is well suited as a probe molecule for [\(^1\)O\(_2\)\textsubscript{OM}] measurements in OM-containing samples because it partitions strongly into DOM due to its high hydrophobicity, and it offers high sensitivity (fM) for \(^1\)O\(_2\) due to its chemiluminescence detection mechanism.

In the present contribution, we prepared model POM consisting of a silica support coated with ad-layers, starting with a positively charged non-photoactive polymer and then with a commercially available humic acid. We compared
photosensitizing properties for the production of $^{1}$O$_{2}$ between model POM and the freely dissolved humic acids. To test the validity of the model POM, analogous experiments were performed with natural particles (suspended lake sediment). Overall, POM showed a similar internal [$^{1}$O$_{2}$]$_{\text{ss}}$ to DOM, and thus was able to efficiently sensitize the photooxidation of a sorbed probe. To our knowledge, this is the first demonstration of a role of POM in the photosensitized production of $^{1}$O$_{2}$.

3.4 Material and methods

Chemicals and General Methods

TPMA and TPMAO$_{2}$ were synthesized as described in the Appendix B. 3-Hydroxybenzaldehyde and n-butyllithium where purchased from Acros Organics. Poly-L-lysine hydrobromide (PLL; 70–150 kDa), used for assembling adlayers, was purchased from Fluka, all the other chemicals were purchased from Sigma-Aldrich. All solvents used for the analysis were chromatography grade. All chemicals were obtained at the highest purity available, and used without further purification unless noted. Solvents for synthesis were dried according to standard purification procedures.$^{54}$ FFA was purified by vacuum distillation prior to use. All aqueous solutions were prepared in ultrapure water (18 MΩ·cm, Barnstead Nanopure Diamond system).

UV-vis absorbance spectra were recorded with a Cary 100 spectrophotometer (Varian). The absorbance of the solution was measured to determine the screening factor of the different samples. (Appendix B)

Synthesis of TPMA

TPMA was synthesized adapting the procedure of Roeschlaub et al.$^{55}$ The Wadsworth-Emmons reaction performed in this work offers numerous advantages
compared to the McMurry coupling reported by Sabelle et al,\textsuperscript{56} and used for the previous synthesis of TPMA by MacManus-Spencer et al\textsuperscript{57} Latch et al\textsuperscript{32} and Grandbois et al.\textsuperscript{43} The advantage of this synthetic procedure is much higher reproducibility of the synthesis, a higher reaction yield, the use of milder conditions that allow an easier scale up of the reaction and less degradation of the vinyl ether to the corresponding ketone. This latter advantage, is especially important since the separation of the vinyl ether from the ketone, during purification is challenging, as the two molecules have similar physical properties. No ketone side-product was observed with the Wadsworth-Emmons procedure, simplifying the final purification. Details of the synthetic procedures and NMR spectroscopic characterization of TPMA can be found in Appendix B.

\textit{Organic matter preparation}

Commercially available humic acid was purchased from Sigma-Aldrich (AHA). The DOM solution was prepared by dissolving AHA in pH 7 phosphate buffer and pH 7 buffered D$_2$O and a 50\% mixture of the two. The AHA solutions were sterile-filtered (0.22 \textmu m) and stored for less than 1 week before the experiments (4 °C, dark). The total organic carbon content (TOC) was determined (Shimadzu Corporation, TOC-L analyzer).

The POM samples were prepared by coating silica microspheres (photochemically inert) with PLL, a photochemically inert and positively charged polymer. The PLL-coated spheres were further coated with AHA as the final top layer. The procedure was adapted from Sander et al.\textsuperscript{58} Briefly, monodisperse silica microspheres of 0.5 and 1 \textmu m nominal diameter were purchased as a suspension in nanopure water, (Bangs Laboratories, Inc., nonporous silica, refractive index of 1.43–1.47 at 589 nm, density 2 g/cm$^3$), giving two POM samples, POM-0.5 and POM-1.0.
All coating steps were performed with phosphate buffer (pH 7, ionic strength (IS) 10 mM sodium chloride). First, the silica particles were suspended at 10 g SiO$_2$/L in buffer. The suspension was added to a solution of PLL (10-fold excess by weight) under sonication at 0 °C, with a constant flow of 200 µL/min controlled by a peristaltic pump. Then, the suspended particle solution was stored overnight at 4 °C (dark). The PLL-coated particles were concentrated by centrifugation, the supernatant was removed, and the particles were resuspended in fresh buffer to remove excess PLL. The resuspension process was facilitated through sonication. The washing procedure was repeated at least five times with fresh buffer. The suspension of PLL-coated particles was concentrated to 10 g SiO$_2$/L, sonicated for 4 h in an ice bath, and then added to a solution of humic acid (5-fold excess by mass in buffer) with a constant flow of 200 µL/min controlled by a peristaltic pump. The suspension of synthetic POM particles was stored at 4 °C in the dark overnight and then washed at least five times following the same procedure described for the PLL coated particles to remove the excess humic acid and then concentrated by centrifugation to 10 g SiO$_2$/L. Finally, the synthetic POM particles were stored at 4 °C in the dark for less than 1 week before use.

The particles were subjected to zeta potential, elemental composition, and TOC analyses to assess the loading of organic matter (Appendix B). The stability of the particles in buffer with respect to loss of organic matter was also verified. The particles were removed from the suspension by centrifugation after overnight storage at 4 °C in the dark. The UV absorbance spectrum of the particle-free buffer showed that no DOM leached from the POM.

The same material (AHA) was used as the organic matter source for both POM and DOM samples to minimize differences in chemical composition in the
organic matter between the samples. The drawbacks of using AHA as a surrogate for natural organic matter have been well documented.\textsuperscript{59,60} Nevertheless, AHA met all of the main requirements of this study, which were that it sorbs organic molecules and produces $^{1}\text{O}_2$ upon irradiation. In addition, its relatively low cost made the significant loss of organic matter in the POM preparation (5-fold excess needed) less of a practical concern. To validate the use of the AHA-based POM model, we compared our results to those using natural sediments obtained from Lake Baldegger (Baldeggersee, CH).

\textit{Photochemical reactions}

Borosilicate tubes (10 mL) containing the reaction solutions were placed in a merry-go-round sample holder inside a photo reactor (Rayonet, Southern New England Ultraviolet Co, 4 × 365 nm bulbs). During irradiation, samples were stirred to avoid sedimentation of the particles, ventilation in the reactor kept the temperature constant within 25–30 °C. The samples contained 100 µM FFA and 10 µM TPMA with 0.001% THF as a cosolvent to favor the dissolution of TPMA. The samples contained either 10 mg$_C$/L AHA (DOM), 10 mg$_C$/L silica coated beads of 0.5 µm diameter (POM-0.5), 10 mg$_C$/L silica coated beads of 1.0 µm diameter (POM-1.0), or a 1:1 mixture (based on OM content) of POM and DOM with a final OM concentration of 20 mg$_C$/L (DOM + POM-0.5; DOM + POM-1.0).

\textit{Singlet oxygen measurements}

\textbf{Aqueous $[^1\text{O}_2]_{solv}$.} The FFA probe concentration was monitored during the reaction by sampling aliquots of the reaction mixtures at specific time-points, and analyzed by HPLC, (Column: Waters, reverse phase C18, eluent: 15% acetonitrile (AcN), 85% pH 5 acetate buffer). The particles were removed before the analysis by
centrifugation. No loss due to sorption to the particles was observed. The aqueous $[^1\text{O}_2]_{ss}$ was calculated using the slope of the $\ln([\text{FFA}]_t/[\text{FFA}_0])$ vs time trace and the rate constant of FFA with $^1\text{O}_2$ ($8.3 \times 10^7 \text{ M}^{-1} \text{s}^{-1}$).\textsuperscript{61}

DOM internal $[^1\text{O}_2]_{ss}$. The production of the dioxetane (TPMAO$_2$) was monitored by triggered luminescence. The procedure was adapted from McManus-Spencer et al.\textsuperscript{57} Briefly, aliquots of the reaction mixture were diluted (1:1000) in dry acetonitrile (AcN). The AcN solution (2 mL) was then exposed to the triggering agent, tetra-$n$-butyl ammonium fluoride (10 µL, 1 M in THF). The luminescence emitted by the solution was measured with a chemiluminometer (Promega, GloMax 20/20). The internal $[^1\text{O}_2]_{ss}$ was obtained using the slope of the [TPMAO$_2$] vs time trace and the rate constant of TPMA with $^1\text{O}_2$ ($1.7 \times 10^6 \text{ M}^{-1} \text{s}^{-1}$).\textsuperscript{57}

3.5 Results and discussion

We assessed the steady-state concentration of $^1\text{O}_2$, $[^1\text{O}_2]_{ss}$, produced from POM-0.5 and POM-1.0 suspensions as well as DOM solutions using FFA for $[^1\text{O}_2]_{aq}$ and TPMA for $[^1\text{O}_2]_{OM}$. The experimental design was such that there were DOM-only solutions (10 mg$_C$/L), POM-only suspensions (10 mg$_C$/L), and mixed DOM-POM suspensions (10 mg$_C$/L DOM + 10 mg$_C$/L POM). The impetus for this design was that the large differences in optical properties (i.e., light screening and light scattering) between the DOM-only and POM-only systems made it impossible to ascribe differences in the $^1\text{O}_2$ measured in the two systems as simply being due to differences in the type of organic matter (dissolved versus particulate). The mixed DOM-POM experiments were used to deconvolute the contributions of optical properties and organic matter type to the overall kinetics.
Another aspect of the experimental design was that the same OM (AHA) was used in the DOM samples and in the preparation of the synthetic POM-0.5 and POM-1.0 samples. The idea behind this choice was to minimize differences in the inherent optical properties, photosensitization efficiencies, and sorption behavior of the OM in the DOM, POM-0.5 and POM-1.0 samples. Nevertheless, it is unknown whether significant differences in the properties of the OM in these samples existed. In fact, it is reasonable to expect that differences could arise either due to selective sorption of certain OM constituents in the preparation of POM-0.5 and POM-1.0 or due to changes in the properties caused by aggregation. Indeed, as discussed below, evidence was obtained that the DOM solutions produced $^1\text{O}_2$ more efficiently than either POM-0.5 or POM-1.0, indicating that there were significant differences in the OM between the particulate and dissolved samples.

The two probes employed in this study were previously used and are known to be specific for the aqueous phase and OM respectively. The FFA and TPMA probe molecules have partition coefficients to organic matter ($\log K_{OM}$) of -0.02 (corresponding to a molar fraction ($f_{OM}$) of 1.25 x10\(^{-5}\)) and 7.1, ($f_{OM}$ of 9.94 x 10\(^{-1}\)) respectively. According to its partition coefficient we expect that FFA is evenly distributed in the solution and measures the average $[^1\text{O}_2]_{\text{ss}}$ over the whole sample volume. The average concentration approaches the concentration in the aqueous phase since the volume occupied by DOM is negligible compared to the volume of the entire solution (for 20 mg/L of AHA, $d = 1.5\text{g/cm}^3$, we expect 13 $\mu\text{L/L}$ to be occupied by OM). By contrast, we expect that TPMA partitions strongly to the organic matter region (99.4%, and only 0.6% in the aqueous solution). The $[^1\text{O}_2]_{\text{ss}}$ measured with TPMA is a reflection of the $^1\text{O}_2$ concentration experienced by
molecules sorbed to OM, and is therefore of relevance for understanding the photochemistry of OM-sorbed species.

The localization of TPMA in the POM and FFA in the aqueous solution was experimentally verified by a photolysis experiment performed in D$_2$O (Appendix B, Table S3.2). Singlet oxygen has a longer lifetime in D$_2$O compared to H$_2$O, which results in enhanced $^1$O$_2$ concentrations in the aqueous fraction, where the main loss process for $^1$O$_2$ is relaxation to its ground state by interaction with the solvent. Therefore, D$_2$O-based enhancement of [$^1$O$_2$]$_{ss}$ measured by a probe indicates the probe is in the aqueous solvent. By contrast, if no solvent isotope effect is observed in probing $^1$O$_2$, this is consistent with the probe being in the OM environment where the composition of the solvent does not affect the local concentration. The primary loss process for $^1$O$_2$ in the OM environment is not relaxation, but diffusive escape into the aqueous solvent. We observed that the [$^1$O$_2$]$_{ss}$ value determined by FFA was enhanced by an average factor of 6 in D$_2$O compared to H$_2$O, confirming that FFA was mostly partitioned in the aqueous phase. A similar result was obtained using H$_2$O/D$_2$O mixtures (discussed below). By contrast, no variation in the [$^1$O$_2$]$_{ss}$ was observed when using TPMA as the $^1$O$_2$ probe, consistent with the localization of TPMA in the organic matter.

_Aqueous-phase $^1$O$_2$ in POM and DOM samples_

The aqueous phase $^1$O$_2$ concentrations produced by DOM- and POM-sensitization differed significantly. In DOM-containing solutions (DOM, POM-0.5+DOM, and POM-1.0+DOM), [$^1$O$_2$]$_{aq}$ as determined by FFA degradation kinetics ranged from 0.48 ± 0.18 to 0.56 ± 0.04 pM. The similar values obtained for these samples indicate that the formation rates of $^1$O$_2$ were also similar. This is an indication that the presence of particles in the POM-0.5+DOM and POM-1.0+DOM
samples did not significantly attenuate the light field experienced by the DOM sensitizer.

By contrast, we observed that the $[^1\text{O}_2]_{\text{aq}}$ detected by FFA in the presence of only POM were on average 6.5 times lower than in the presence of DOM (Table 3.1, Figures 3.1 and 3.2). Similar $[^1\text{O}_2]_{\text{aq}}$ values were obtained for the synthetic (POM-0.5 and POM-1.0) and natural Baldeggersee sediment samples (details on the material are reported in the Appendix B). The low $[^1\text{O}_2]_{\text{aq}}$ measured by FFA in the presence of particles is consistent with prior reports.\(^{14}\) If the particles have little effect on the internal light field, as indicated in the experiments noted above, then the lower steady-state concentrations of $^1\text{O}_2$ suggest lower production rates of $^1\text{O}_2$ by POM.

Figure 3.1 also shows the effect of added D$_2$O on the OM-sensitized degradation of FFA. The 2-fold increase of the $[^1\text{O}_2]_{\text{ss}}$ detected in the H$_2$O/D$_2$O 1:1 mixture, for all the sensitzers tested, indicates that the FFA degradation was due to $^1\text{O}_2$ reaction in the aqueous solvent. Dark control experiments showed no reaction occurring without irradiation and no loss of FFA due to sorption to the particles.

\textbf{Figure 3.1:} Sensitized FFA degradation in the presence of DOM and POM. The kinetics of FFA photooxidation are shown under different conditions (buffered H$_2$O = blue circles; buffered D$_2$O/H$_2$O 1:1 = red triangles; and, buffered H$_2$O without irradiation = black squares). (left panel) DOM, (central panel) POM-1.0, and (right panel) POM-1.0 and DOM.
Figure 3.2. Kinetics of FFA photooxidation with different sensitizers. Different marker shapes represent different sensitizers: Black open circles, no sensitizers; black filled circle, DOM; red squares, POM-0.5; and, blue triangles, POM-1.0. The linear regressions reported in these panels are weighted by the uncertainty of the individual points.
Table 3.1. Singlet oxygen steady state concentrations ([O$_2$]$_{ss}$) upon irradiation of POM, DOM and POM/DOM mixtures in different portions of the solution, reported in picomolar (pM) units

<table>
<thead>
<tr>
<th>TOC (mg/L)</th>
<th>Sensitizer</th>
<th>DOM (AHA)</th>
<th>POM 0.5</th>
<th>POM 1.0</th>
<th>DOM + POM 0.5</th>
<th>DOM + POM 1.0</th>
<th>Baldeggersee Sediment</th>
</tr>
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<tr>
<td>[O$<em>2$]$</em>{ss}$ uncorrected</td>
<td>Internal$^a$</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Aqueous$^b$</td>
<td>26±2</td>
<td>36±1</td>
<td>30±1</td>
<td>31±2</td>
<td>31±2</td>
<td>34±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.41±0.02</td>
<td>0.07±0.04</td>
<td>0.10±0.05</td>
<td>0.40±0.02</td>
<td>0.47±0.04</td>
<td>0.05±0.07</td>
</tr>
<tr>
<td>[O$<em>2$]$</em>{ss}$ screening corrected</td>
<td>Internal$^a$</td>
<td>31±2</td>
<td>-</td>
<td>-</td>
<td>37±2$^d$</td>
<td>38±2$^d$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aqueous$^b$</td>
<td>0.49±0.03</td>
<td>-</td>
<td>-</td>
<td>0.48±0.18$^d$</td>
<td>0.56±0.04$^d$</td>
<td>-</td>
</tr>
<tr>
<td>Internal/Aqueous$^c$</td>
<td>64±7</td>
<td>520±280</td>
<td>300±140</td>
<td>76±6</td>
<td>67±7</td>
<td>680±410</td>
<td></td>
</tr>
</tbody>
</table>

$^a$[O$_2$]$_{ss}$ measured using a hydrophobic probe (TPMA), $^b$[O$_2$]$_{ss}$ measured using a hydrophilic probe (FFA), $^c$ ratio of the [O$_2$]$_{ss}$ measured with TPMA and FFA. $^d$ Data corrected only for DOM (AHA) screening.
OM-phase \(^{1}\text{O}_2\) in POM and DOM samples

Steady-state concentrations of \(^{1}\text{O}_2\) in the OM-phase were measured by TPMA following the formation of the oxidation product (TPMAO\(_2\)) by chemiluminescence-based detection (Figure 3.3). High \([^{1}\text{O}_2]_{\text{OM}}\) values were obtained for all OM-containing samples, ranging from 31 ± 2 to 38 ± 2 pM (Table 3.1). The higher \([^{1}\text{O}_2]_{\text{OM}}\) compared to \([^{1}\text{O}_2]_{\text{aq}}\) in the DOM (AHA) sample reflects trends observed in previous studies.\(^{32,43}\) TPMA also experienced high \(^{1}\text{O}_2\) concentrations in suspensions of synthetic and natural POM (Table 3.1).

![Figure 3.3. TPMA photo-oxidation with different sensitzers.](image)

Comparing the \([^{1}\text{O}_2]_{\text{OM}}\) values between POM and DOM solutions, the \([^{1}\text{O}_2]_{\text{OM}}\) values were up to 30% higher in POM than in DOM solutions (Table 3.1). This result supports the hypothesis that POM could play an important role in the photochemistry of POM-sorbed compounds. Looking more closely at the data, we observed two interesting results. First, the ratio of \([^{1}\text{O}_2]_{\text{OM}}\) and \([^{1}\text{O}_2]_{\text{aq}}\) is about 8 times larger for POM than for DOM. Second, the \([^{1}\text{O}_2]_{\text{OM}}\) values for POM were up to 30% higher.
than for DOM. The second result might be considered surprising, given that the source of OM was the same for both POM and DOM samples (both AHA). A model that rationalizes the distribution of $^1\text{O}_2$ in the various samples is discussed in the next section.

Note that the values for the POM samples reported in Table 3.1 were uncorrected for light screening. This is due to the difficulties in quantifying the screening in the particle solution. The light attenuation measured by the collimated beam in a UV–vis absorption spectrometer using a standard 1 cm cuvette was ca. 85%. However, scattered light that does not strike the detector is measured as “attenuation” but can initiate a photochemical reaction in another part of the sample where it has been redirected. To test this aspect, we performed the photolysis by irradiation of samples containing a mixture of DOM and POM. If the presence of the particles would lead to significant light attenuation, the $[^1\text{O}_2]_{\text{aq}}$ would be lower in the POM+DOM samples compared to the DOM sample. Note that even though in the POM+DOM samples there is double the amount of OM in the sample, no significant enhancement is expected for both $[^1\text{O}_2]_{\text{aq}}$ and $[^1\text{O}_2]_{\text{OM}}$ compared to DOM. In the aqueous phase, no enhancement is expected in $[^1\text{O}_2]_{\text{aq}}$ for POM+DOM compared to DOM because the contribution of POM to $[^1\text{O}_2]_{\text{aq}}$ is not significant. In the OM phase, it is expected that the TPMA probe is fully OM-bound in the POM+DOM and DOM samples. 32, 43

Thus, no enhancement in the $[^1\text{O}_2]_{\text{OM}}$ observed by TPMA is expected. The similarity of the $[^1\text{O}_2]_{\text{aq}}$ values in the DOM and POM-0.5+DOM and POM-1.0+DOM samples suggests that the suspended particles in the POM samples did not lead to significant light attenuation. This hypothesis was verified performing actinometrical measurements in the presence of coated and uncoated particles (Appendix B).
experiment confirms that the screening effect is compensated by an increase of light pathlength due to scattering.

$^1O_2$ distribution in various samples

To understand the distribution of $^1O_2$ in the POM and DOM samples, we interpreted the data using a conceptual model that separates the system into two compartments: OM and aqueous. In this model (Scheme 3.1), all of the $^1O_2$ is initially formed in the OM compartment. There are two possible loss processes occurring: the quenching by the DOM itself and the diffusion in the aqueous phase. Essentially, all of the $^1O_2$ that is not quenched escapes into the aqueous compartment, where relaxation by the solvent is the dominant loss process. The two compartments have very different volumes: 13 $\mu$L_{OM}/L_{tot} (for 20 mg/L AHA solution (AHA density =1.5 g/cm$^3$) and 1 L_{aq}/L_{tot} ($V_{aq} \sim V_{tot}$).

![Scheme 3.1: Kinetic scheme of $^1O_2$ formation and loss in an OM containing system. $^1O_2$ is formed inside the OM aggregate at a specific rate of formation ($R_f$). The loss processes in the OM are diffusion from the DOM, with rate constant $k_{diff}$, and quenching by the DOM itself with a bimolecular rate constant of quenching ($k_{OM}$) dependent on the concentration of the quencher ([OM]).](image)

For the kinetic model presented in Scheme 3.1, the steady-state concentration of $^1O_2$ in the OM compartment, $[^1O_2]_{OM}$, is given by equation 3.1, where $R_f$ is the rate
of formation in the OM compartment, $k_{OM}$ is the quenching rate constant of $^{1}\text{O}_2$ by OM, $[\text{OM}]_{OM}$ is the concentration of the OM in the OM compartment, and $k_{diff}$ is the rate constant for loss of $^{1}\text{O}_2$ from the OM compartment by diffusion.

$$[^{1}\text{O}_2]_{OM} = \frac{R_f}{k_{OM}[^{1}\text{O}_2]_{OM}+k_{diff}}$$  \hspace{1cm} (3.1)

The simplified expression\textsuperscript{43} for the steady-state concentration of $^{1}\text{O}_2$ in the aqueous phase, $[^{1}\text{O}_2]_{aq}$, is given by equation 3.2.

$$[^{1}\text{O}_2]_{aq} = \frac{k_{diff}[^{1}\text{O}_2]_{OM}V_{OM}}{k_{solv}V_{tot}}$$  \hspace{1cm} (3.2)

The source of $[^{1}\text{O}_2]_{aq}$ is the $^{1}\text{O}_2$ that diffuses from the OM compartment to the aqueous compartment, given by $[^{1}\text{O}_2]_{OM}$ times $k_{diff}$ scaled by the ratio of the volume occupied by the OM, $V_{OM}$, to the total volume $V_{tot}$. The rate constant $k_{solv}$ is the solvent-dependent relaxation rate constant for $^{1}\text{O}_2$. Substituting the expression of $[^{1}\text{O}_2]_{OM}$ in equation 3.1 into equation 3.2, we obtain equation 3.3.

$$[^{1}\text{O}_2]_{aq} = \frac{k_{diff}R_fV_{OM}}{k_{solv}(k_{OM}[^{1}\text{O}_2]_{OM}+k_{diff})}$$  \hspace{1cm} (3.3)

Combining equations 3.1 and 3.3, an expression for $k_{diff}$ can be written (equation 3.4).

$$k_{diff} = \frac{k_{solv}V_{tot}}{[^{1}\text{O}_2]_{OM}}$$  \hspace{1cm} (3.4)

From the measured $[^{1}\text{O}_2]_{aq}$ and $[^{1}\text{O}_2]_{OM}$ values for the POM and DOM samples reported in Table 3.1, we obtained rate constants for diffusive loss of $^{1}\text{O}_2$ from the OM compartment in each sample. For POM-1.0, $k_{diff}^{POM}$ equaled $6.4 \times 10^7$ s$^{-1}$, while the analogous rate constant for DOM, $k_{diff}^{DOM}$, was 4.7 times larger, $3.0 \times 10^8$ s$^{-1}$. The higher rate constants for loss of $^{1}\text{O}_2$ from the OM compartment in the DOM samples compared to the POM samples is consistent with the smaller size and consequent larger surface area of the DOM aggregates compared to the POM aggregates.
The photochemical rate of formation of $^1\text{O}_2$, $R_f$, can be estimated for each system by substituting into Equation 3.1 the $k_{\text{diff}}$ value calculated above, but one needs to first estimate the magnitude of the $k_{\text{OM}} \times [\text{OM}]_{\text{OM}}$ term. The $k_{\text{OM}}$ value for AHA is not known, but we estimated it to be $10^6 \text{ M}^{-1} \text{ s}^{-1}$, based on values for Suwannee River and Pony Lake fulvic acids, $4.1 \times 10^5$ and $1.6 \times 10^6 \text{ M c}^{-1} \text{ s}^{-1}$, respectively. The value for $[\text{OM}]_{\text{OM}}$ is in the range of 32–69 M, calculated using the elemental composition of AHA and the density range of 0.7–1.5 g/cm$^3$. Taken together, these values give a pseudo-first-order quenching rate constant for $^1\text{O}_2$ of $3–7 \times 10^7 \text{ s}^{-1}$. Comparing this to the $k_{\text{diff}}$ values for DOM and POM-1.0 above, we see that quenching of $^1\text{O}_2$ by OM is significantly smaller than the diffusional loss of $^1\text{O}_2$ from the OM compartment for DOM. This leads to a calculated rate of $^1\text{O}_2$ formation in the DOM system, $R_f^{\text{DOM}}$, of 7.9 mM s$^{-1}$. For POM-1.0, the two loss processes are competitive, and we can estimate $R_f^{\text{DOM}}$ to be in the range of 2.9 mM s$^{-1}$ (for $[\text{OM}]_{\text{OM}} = 32$ M) to 4.0 mM s$^{-1}$ (for $[\text{OM}]_{\text{OM}} = 69$ M). The rate of formation of $^1\text{O}_2$ for the POM samples is lower than for the DOM samples. Below, we consider the origin of the lower $R_f$ value for the POM samples compared to the DOM samples.

We prepared the synthetic POM samples using a SiO$_2$ support with AHA for the express purpose of minimizing the differences in optical and photochemical properties of the DOM and POM samples. The results obtained in the study indicate that the absorbance properties, the quantum yields, or both, changed upon sorption of AHA to form the POM samples. There are different possible explanations for a lower formation rate of $^1\text{O}_2$ of POM compared to DOM, such as (1) higher light screening in the POM solutions, (2) a geometrical effect due to $^1\text{O}_2$ heterogeneous distribution, and (3) a lower intrinsic quantum yield of POM compared to DOM.
The first possible explanation, that the light absorption of the DOM solution was lower than the light absorption of the POM solution, was investigated. We performed experiments using both DOM and POM in the same solution (POM-0.5+DOM and POM-1.0+DOM). Note that in these experiments there was twice the amount of OM in solution (20 mgC/L), of which, half was immobilized on the particles (POM) and half was free in solution (DOM). If the \([1^\text{O}_2]_{\text{aq}}\) observed with this mixture of POM and DOM was lower than the \([1^\text{O}_2]_{\text{aq}}\) detected in the DOM solution, we can conclude that the low \([1^\text{O}_2]_{\text{aq}}\) was due to light screening. Rather, we observed that the \([1^\text{O}_2]_{\text{aq}}\) values in the mixed samples were similar (actually slightly higher) than in the DOM sample (Table 3.1). These data suggest that the screening effect is not significant. Actinometry experiments in the presence of coated particles and uncoated particles confirm that the screening effect is compensated by the increased path length of light in the sample due to scattering and cannot explain the difference in the \([1^\text{O}_2]_{\text{aq}}\) observed between POM and DOM solutions (Appendix B).

The second possible explanation is a geometrical effect, in which low rates of FFA degradation can be explained by the fact that on average very few FFA molecules are close enough to a POM particle to react with the \(^1\text{O}_2\) generated. This explanation can also be excluded easily. If one considers that \(^1\text{O}_2\) can diffuse only short distances (250 nm) before it is completely quenched by water, and that POM suspensions have the OM condensed in relatively fewer units than in DOM solutions, it is true that the fraction of the total system volume where \(^1\text{O}_2\) is accessible to FFA is much smaller in the case of POM compared to DOM. However, the concentration of \(^1\text{O}_2\) in this volume is also significantly higher in the case of POM compared to DOM (for the same quantum yield of POM and DOM). There are end-member extreme scenarios where the heterogeneous distribution of \(^1\text{O}_2\) can affect the FFA
measurement of the average $[^1\text{O}_2]_{ss}$ in solution (e.g., if the FFA is consumed faster in the $^1\text{O}_2$ corona surrounding the OM than it can be replaced by diffusion from the aqueous solution). We calculated the [FFA] gradient from the outside to the inside of the OM region (Appendix B). The calculation shows that under the experimental conditions the difference of [FFA] outside and inside DOM is insignificant ($\Delta[\text{FFA}] = 10^{-16}\text{ M}$).

The third possible explanation of a lower intrinsic quantum yield of POM compared to DOM is reasonable, despite the fact that the same AHA source material was used for both the DOM and POM samples. There are at least two reasons for a decrease in the quantum yield of $^1\text{O}_2$ formation going from dissolved AHA to sorbed AHA. First, the sorption of AHA on PLL-coated silica beads could have resulted in the selection of a subset of molecular components that are poor sensitizers for $^1\text{O}_2$. Because the particle preparation method is based on sorption through electrostatic interaction, it is certainly possible, even likely, that fractionation of AHA occurred during the POM preparation. It is also likely that the sorbed molecules are biased toward the larger components of DOM. All studies agree that the small size fractions are the best sensitizers for $^1\text{O}_2$. Second, there may be enhanced relaxation of the $^3\text{CDOM}$ (precursor of $^1\text{O}_2$) when sorbed. Such enhanced relaxation may be due to aggregation of the OM molecules (self-quenching) or by interaction with the amine-rich PLL interlayer.

Pulling together the above calculations and discussion, we can summarize the situation as follows. The synthetic and natural POM samples are poorer photosensitizers than the DOM samples examined, producing $^1\text{O}_2$ at a lower apparent rate, this being either due to lower quantum yield of POM compared to DOM, or due to the more significant quenching of $^1\text{O}_2$ by OM in the POM sample compared to
DOM. This results in POM being a poor photosensitizer for dissolved species such as FFA. However, sorbed compounds experience similar $^1\text{O}_2$ concentrations in both the POM and DOM systems, due to the fact that the decreased production of $^1\text{O}_2$ in the POM systems is compensated by a slower diffusive loss of $^1\text{O}_2$ from the OM microphase.

### 3.6 Environmental implications

Using a dual probe approach, we were able to demonstrate the photoactivity of POM in the production of $^1\text{O}_2$. Synthetic POM particles appear to simulate well the photochemical properties of at least one sample of natural sediment particles. The $^1\text{O}_2$ concentration experienced by a probe sorbed to POM is similar or higher than for one sorbed to DOM. Thus, we expect that particles play a key role in the photodegradation of particle-bound organic pollutants and inactivation of sorbed bacteria and viruses. Thus far, these POM-sensitized processes have mostly been neglected in photochemical studies but will be relevant for various environmental systems where a significant fraction of the organic matter is associated with particles. In such systems, adsorbed pollutants or pathogens can experience much higher $^1\text{O}_2$ than what is found in the aqueous solution. The results of this study indicate that POM is not an important sensitizer for dissolved species.

The effect of removing POM on the apparent $^1\text{O}_2$ concentration, $[^1\text{O}_2]_{\text{app}}$, experienced by a compound is dependent on the compound’s affinity for organic matter. This is graphically depicted in **Figure 3.4**, in which the effect of removing particles on $[^1\text{O}_2]_{\text{app}}$ is plotted versus the compound’s log $K_{oc}$ value. The function has an inverted bell shape with a minimum at a log $K_{oc}$ value near 4. The reason for this shape is that at low log $K_{oc}$ values, only aqueous phase $^1\text{O}_2$ is important and removal
of POM causes only a small perturbation to this pool of $^{1}$O$_2$. At high log $K_{OC}$ values, essentially all of the compound is OM-bound, and removal of POM causes only a small perturbation to the OM-bound fraction. At intermediate values of log $K_{oc}$, when the OM-bound fraction is large enough that there is a strong microheterogeneous effect but small enough that removing POM from the system significantly changes the OM-bound fraction, the maximum effect of removing POM is predicted. The magnitude of the effect is proportional to the POM:DOM carbon ratio and is illustrated in Figure 3.4 for four different POM:DOM ratios.

**Figure 3.4.** Effect of particle removal on $^{1}$O$_2$ exposure. The apparent $^{1}$O$_2$ concentration, $[^{1}$O$_2]_{app}$, experienced by a compound in a filtered solution normalized to $[^{1}$O$_2]_{app}$ for the analogous unfiltered solution plotted as a function of the compound’s $K_{OC}$ value. The functions were calculated assuming 10 mg C/L total OM with POM representing 5, 10, 30, and 50% of the total OM concentration, $[^{1}$O$_2]_{POM} = 33$ pM, $[^{1}$O$_2]_{DOM} = 26$ pM, $[^{1}$O$_2]_{aq,filtered} = 0.04$ pM (mg C/L)$^{-1}$ $\times$ [DOM], and $[^{1}$O$_2]_{aq,unfiltered} = 0.04$ pM (mg C/L)$^{-1}$ $\times$ [DOM] + 0.008 pM (mg C/L)$^{-1}$ $\times$ [POM]. The approximate positions of FFA,$_{32}$ naphthalene, anthracene, tetracene,$_{65}$ and TPMA$_{32}$ on the curve are shown.
We believe that further work is necessary in the direction of a systematic study of natural particles of different source and composition. Moreover an extension of the study of POM photochemistry to other PPRI is also needed.

3.7 Acknowledgements

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3.8 References


3.9 Appendix B: Supporting material for Chapter 3

Synthesis of 3-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)phenoxy)(tert-butyl)dimethylsilane (TPMA)

Scheme S3.1. Synthesis overview
Synthesis of 3-(tert-butylidimethyl)siloxybenzaldehyde (1)

The procedure was adapted from Bastos et al. A CEM Peptide Hydrolysis Discover Microwave was used as a source of radiation. Hydroxylbenzaldehyde (6.12 g, 50 mmol, 1 equiv), tert-butylidimethylsilyl chloride (9.05 g, 60 mmol, 1.2 equiv) and imidazole (10.21 g, 150 mmol, 3 equiv) were placed in a round bottom flask and exposed to three microwave radiation cycles. The power was set to 45 W and the maximum temperature to 125 °C. The reaction mixture was allowed to cool between cycles and the reaction was monitored by TLC (1:10 EtOAc:n-hexane). After three cycles, the reaction mixture was allowed to stand overnight. The reaction mixture was washed with water (1 × 50 mL) and the product extracted with EtOAc (3 × 50 mL). The organic phase was dried with MgSO₄ and then the solvent removed by rotary evaporation. The crude product following extraction contained ca. 10% impurity, as determined by ¹H NMR spectroscopy. The product was purified by flash chromatography (SiO₂, 1:10 EtOAc:n-hexane). The purified product was isolated in 87 % yield ¹H-NMR (400 MHz, CDCl₃) δ: 0.12 (s, 6H MeSi), 0.90 (s, 9H t-Bu), 7.11 (ddd, J=1.18 Hz, 2.46 Hz, 8.1 Hz, 1H, Ar), 7.33 (dd, J=2.21 Hz, 1.67 Hz, 1H, Ar), 7.40 (t, J=7.7 Hz, 1H, Ar),
7.47 (dt, J=1.3 Hz, 7.6 Hz; 1H, Ar), 9.95 (s, 1H, HCO). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: -4.28, 18.35, 25.76, 120.03, 123.70, 126.70, 130.23, 138.09, 156.56, 192.26.

(tert-buty1(3-(dimethoxymethyl)phenoxy)dimethylsilane) (2)

Scheme S3.3. Synthesis of (tert-buty1(3-(dimethoxymethyl)phenoxy)dimethylsilane)

The synthesis of 2 was adapted from Roeschlaub et al.$^2$ Compound 1 (3 g, 12.7 mmol, 1 equiv) was dissolved in 2,2-dimethoxypropane (3.2 mL, 25.8 mmol, 2 equiv) and few crystals of dry p-toluenesulfonic acid were added. The reaction mixture was gently heated to 70 °C while the acetone formed was distilled off the mixture and heating continued until no more acetone was formed. The reaction was cooled to room temperature, quenched with aq. NaHCO$_3$ (50 mL) and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The organic phase was washed with water (1 × 50 mL) and dried over MgSO$_4$. The solvent was removed by rotary evaporation. The product was purified by vacuum distillation. The product (2) (99% purity by NMR) was isolated in 52% yield. $^1$H-NMR (400 MHz, CDCl$_3$) δ 0.19 (6H, s, MeSi), 0.98 (9H, s, t-Bu) 3.32 (6 H, s, MeO), 3.49 (1 H, s, ArCH), 6.80 (1 H, ddd, J 0.8 Hz, 2.4 Hz, 8 Hz, ArH), 6.93(1 H, t, J 1.96 Hz, ArH), 7.04 (1 H, d, J 7.68 Hz ArH), 7.22 (1 H, t, J 7.9 Hz, ArH). 13C-NMR (100 MHz, CDCl$_3$) δ: -4.29, 18.33, 25.82, 52.77, 103.2, 116.70, 120.22, 123.68, 129.29, 139.76, 155.77.
**Dimethyl ((3-(tert-butyldimethylsiloxy)phenyl)(methoxy)methyl)-phosphonate (3)**

**Scheme S4.** Synthesis of dimethyl ((3-((tert-butyldimethylsilyl)oxy)phenyl)(methoxy)methyl)phosphonate

The synthesis was adapted from Roeschlaub et al.\(^2\) Compound 2 (2.4 g, 8.09 mmol, 1 equiv) and trimethylphosphite (1.4 mL, 12.35 mmol, 1.5 equiv) were dissolved in CH\(_2\)Cl\(_2\) (15 mL, 32 equiv) and cooled to -84 °C under dry N\(_2\) atmosphere. TiCl\(_4\) (1.4 mL, 12.8 mmol, 1.5 equiv) were added dropwise by addition funnel over 30 min. The reaction mixture was stirred for 30 min at -84 °C and then allowed to warm to room temperature and further stirred for 45 min. The reaction mixture was quenched with MeOH:H\(_2\)O 2:1. The organic phase was extracted with CH\(_2\)Cl\(_2\) (3 × 25 mL), washed with aq. NaHCO\(_3\) (1 × 25 mL) and brine (1 × 50 mL), and then dried over Na\(_2\)SO\(_4\). The solvent was removed by rotary evaporation. The product was purified by flash chromatography (EtOAc: petroleum ether 3:1) and isolated in 92% yield. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.20 (6H, s, MeSi), 0.98 (9H, s, t-Bu) 3.38 (3 H, s, MeO), 3.72 (6 H, dd, J 7, 10Hz, MeOP), 4.60 (1 H, d, J 15 Hz, CHP), 6.82 (1 H, dddd, J 1, 2.6, 8.1 Hz,ArH), 6.95 (1 H, dd, J 2.2, 4.1,ArH), 7.02 (1 H, d, J 7.7, ArH), 7.24 (1 H, t, J 7.9 Hz, ArH). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) \(\delta\) -4.31, 18.37, 25.82, 58.82, 81.02, 119.78, 120.60, 121.28, 129.78, 135.71, 156.02.
Diisopropylamine (1.62 mL, 11.28 mmol, 1.6 equiv) was dissolved in THF (9 mL) under dry N₂ atmosphere, and cooled to -84 °C. n-Butyllithium (7.05 mL, 11.28 M in hexanes, 1.6 equiv) was added dropwise and the mixture was stirred for 30 min to allow complete conversion to LDA. Compound 3 (2.54 g, 7.05 mmol, 1 equiv) dissolved in THF (10 mL) was added at -84 °C dropwise by syringe over 15 min. The mixture was stirred for 1 h. Adamantanone (1.05 g, 7.05 mmol, 1 equiv) was dissolved in THF (3.5 mL) and added dropwise to the reaction mixture by syringe over 30 min. The reaction mixture was allowed to warm to room temperature and stirred for 6 h. The reaction was quenched with pH 7 phosphate buffer 0.2 M (25 mL), and the organic phase was rapidly extracted with CH₂Cl₂ (3 × 50 mL). The extract was washed with aq. NaHCO₃ (2 × 50 mL) and then with cold brine (2 × 50 mL). The organic phase was dried over MgSO₄ and the solvent was removed by rotary evaporation. The product was purified by flash chromatography (19:1 petroleum ether: EtOAc) The purified product was recovered in 65% yield. ¹H-NMR (400 MHz, CDCl₃) δ 0.20 (6H, s, MeSi), 0.98 (9H, s, t-Bu), 1.78–1.97 (12 H, m, adamantanyl-H), 2.63 (1 H, br s, adamantanyl-H), 3.24 (1 H, br s, adamantanyl-H), 3.29 (3 H, s, MeO), 6.78 (2 H, tdd, J 0.9 Hz, 2.4 Hz, 8.05 Hz, ArH), 6.91 (1 H, dt, J 1.2 Hz, 7.6 Hz, ArH), 7.20 (1 H, t, J 7.8 Hz, ArH), 13C-NMR
tert-butyldimethyl(3-((1r,3r,5r,7r)-spiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)phenoxy)silane

Scheme S3.6. Dioxetane synthesis

TPMA (150 mg, 0.4 mmol) was dissolved in a solution of methylene blue in CDCl$_3$ (10 µM, 2 mL) and placed in an NMR tube. The solution was cooled to 0 °C in an ice bath and irradiated with a xenon lamp with a 455 nm cutoff filter. The solution was bubbled with synthetic air and the product formation was followed by $^1$H NMR. After 5 hours the $^1$H NMR spectrum showed complete consumption of the starting material and formation of two products. The reaction mixture was filtered through a plug of silica to remove methylene blue. The silica was washed with CH$_2$Cl$_2$. The combined organic filtrates were purified by flash column (2:1 CH$_2$Cl$_2$:n-hexane). The pure product was recovered in 38% yield.$^1$H-NMR (400 MHz, CDCl$_3$) NMR δ 0.19 (6H, s, MeSi), 0.99 (9H, s, t-Bu), 1.45–1.94 (12 H, m, adamantanyl-H), 2.24 (1 H, br s, adamantanyl-H), 3.02 (1 H, br s, adamantanyl-H), 3.24 (3 H, s, MeO), 6.88 (2 H, dd, $J$ 9.23 Hz, 2.35 Hz, ArH), 7.29 (2 H, t, $J$ 7.8 Hz, ArH). $^{13}$C-NMR (100 MHz, CDCl$_3$)
Title: TPMA PROTON in CDCl3

| ppm | 7.4 | 7.3 | 7.2 | 7.1 | 7.0 | 6.9 | 6.8 | 6.7 | 6.6 | 6.5 | 6.4 | 6.3 | 6.2 | 6.1 | 6.0 | 5.9 | 5.8 | 5.7 | 5.6 | 5.5 | 5.4 | 5.3 | 5.2 | 5.1 | 5.0 | 4.9 | 4.8 | 4.7 | 4.6 | 4.5 | 4.4 | 4.3 | 4.2 | 4.1 | 4.0 | 3.9 | 3.8 | 3.7 | 3.6 | 3.5 | 3.4 | 3.3 | 3.2 | 3.1 | 3.0 | 2.9 | 2.8 | 2.7 | 2.6 | 2.5 | 2.4 | 2.3 | 2.2 | 2.1 | 2.0 | 1.9 | 1.8 | 1.7 | 1.6 | 1.5 | 1.4 | 1.3 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

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DE               6.50 usec
TE            300.0 K
D1      1.00000000 sec

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WDW              E
MM
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GB               0
PC              1.00
Particles characterization

The organic carbon content was measured by TOC analyzer and is summarized in Table S3.1.

### Table S3.1. Carbon content in the different particles samples

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<th></th>
<th>POM-0.5</th>
<th>POM-1.0</th>
<th>N-POM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC$^a$ content</td>
<td>0.89%</td>
<td>0.49%</td>
<td>1.62%</td>
</tr>
<tr>
<td>IC/PLL$^b$</td>
<td>0.6%</td>
<td>0.3%</td>
<td>8.06%</td>
</tr>
<tr>
<td>TC$^c$</td>
<td>1.5%</td>
<td>0.85%</td>
<td>9.68%</td>
</tr>
</tbody>
</table>

$^a$ Organic carbon, $^b$ Inorganic carbon and PLL derived carbon, $^c$ Total carbon

The synthetic particles were further analyzed by zeta potential, to assess that the surface charge was changing from negative (SiO$_2$) to positive (PLL coated SiO$_2$) back to negative (AHA coated PLL-coated SiO$_2$).

Baldeggersee sediment preparation details

A sediment sample from Baldeggersee was collected, washed with nanopure water three times and filtered through a 0.5 μm filter. The sediment retained by the filter was dried under vacuum and further used for the photolysis experiment.
Screening correction calculation

Rate constants obtained from experiments with DOM solutions and DOM+POM samples were corrected for light screening. The absorption spectra were measured in 1 cm quartz cuvettes using a Cary 100 spectrophotometer (Varian) for solutions containing DOM (10 mg C/L), POM (10 mg C/L) and DOM+POM. The relative irradiance, $E_\lambda$, of the UV light was recorded with a spectrometer (OceanOptics Inc.). The screening factor, $S$, is described in Equation S3.1, where $\langle \rangle$ is the optical density at each wavelength, $\lambda$ (nm), and $z$ (cm) is the optical path length.

$$S_\lambda = \frac{1 - 10^{\langle \rangle_{\lambda} z}}{2.303 \alpha_{\lambda} z} \quad (S3.1)$$

The relative light intensity experienced by OM was estimated as follows,

$$I = \sum_\lambda S_\lambda \times E_\lambda \quad (S3.2)$$

where $E_\lambda$ is the relative light intensity of the lamp at each wavelength. Light screening was calculated for the wavelength range in which chromophores DOM absorb light (345-410 nm). The observed degradation rates of the probes were corrected for light screening by dividing the measured value by the estimated $I$ value.

Table S3.1 summarizes the relative light intensity experienced for each sample. The POM-containing samples had a light attenuation factor far larger than 50% (and thus were not considered quantitatively useful as they led to correction factors that were larger than the original value).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$I$</th>
<th>Attenuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.98</td>
<td>2%</td>
</tr>
<tr>
<td>DOM</td>
<td>0.83</td>
<td>17%</td>
</tr>
<tr>
<td>POM 0.5</td>
<td>0.17</td>
<td>84%</td>
</tr>
<tr>
<td>POM 1</td>
<td>0.16</td>
<td>84%</td>
</tr>
<tr>
<td>POM+DOM 0.5</td>
<td>0.16</td>
<td>85%</td>
</tr>
<tr>
<td>POM+DOM 1</td>
<td>0.15</td>
<td>85%</td>
</tr>
</tbody>
</table>
Partitioning of the two probes (D$_2$O experiment)

According to the $K_{OC}$ of TPMA and FFA, we expect TPMA to partition completely in the DOM and FFA to be evenly distributed in the solution. To verify this model we performed photolysis experiments in D$_2$O and in H$_2$O (with 1% d$_6$-ethanol and ethanol, respectively). Enhancement in the [$^1$O$_2$]$_{aq}$ in the D$_2$O experiment was expected since relaxation by the solvent is the major quenching mechanism for $^1$O$_2$ in aqueous solution. On the contrary, no enhancement was expected for [$^1$O$_2$]$_{OM}$, since the main loss pathway is due to diffusion that is substantially unchanged from D$_2$O to H$_2$O. If TPMA is completely partitioned in DOM we expect unchanged [$^1$O$_2$]$_{ss}$ measured by TPMA in D$_2$O and H$_2$O. Table S3.2 reports the [$^1$O$_2$]$_{ss}$ observed by TPMA and FFA in the two solvents and reflects our expectation concerning the partitions of the probes. Notice that the expected $^1$O$_2$ lifetime enhancement for pure D$_2$O vs pure H$_2$O is 13-fold,$^3$ but the enhancement is sensitive to H$_2$O traces (dropping in half in the presence of 6% H$_2$O),$^3$ and in the experiment design there are several factors that can affect the $^1$O$_2$ lifetime such as the presence of a co-solvent in both the experiments, the presence of water associated with the particles which, for experimental reasons, cannot be dried. In addition the error associated with the FFA measurements in the presence of particles is large due to the uncertainties introduced by the heterogeneity of the system that makes the quantification of the enhancement challenging.
Table S3.2. Solvent isotope effect for TPMA and FFA with different sensitizers

<table>
<thead>
<tr>
<th></th>
<th>[^1\text{O}_2\text{ss}] (\times 10^{-12} \text{ M})</th>
<th>POM-0.5</th>
<th>POM-1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{D}_2\text{O})</td>
<td>27 ± 3.1</td>
<td>25 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>(\text{H}_2\text{O})</td>
<td>25 ± 3.5</td>
<td>33 ± 3.28</td>
</tr>
<tr>
<td></td>
<td>(\text{D}_2\text{O}/\text{H}_2\text{O})</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>FFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{D}_2\text{O})</td>
<td>0.18 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(\text{H}_2\text{O})</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(\text{D}_2\text{O}/\text{H}_2\text{O})</td>
<td>7.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

\(^a\)The \[^1\text{O}_2\text{ss}\] measured by TPMA and FFA are reported in pM concentrations. The ratio between the \[^1\text{O}_2\text{ss}\] detected in the experiment performed in \(\text{D}_2\text{O}\) and the \[^1\text{O}_2\text{ss}\] detected in the experiment performed in \(\text{H}_2\text{O}\) is also reported.
Change of the light intensity in the presence of different sensitizers measured by PNA-Py actinometer

The p-nitroanisole / pyridine (PNA / Py) actinometer was developed by Dulin and Mill and have been widely employed in the environmental photochemistry because it is not sensitive to wavelength or temperature. We can use this actinometer to estimate the enhancement in irradiance in the presence of particles.

![Scheme S3.7. p-nitroanisole-pyridine actinometer reaction](image)

The reaction is the photochemical substitution of pyridine for nitrite. Since it is a bimolecular reaction, the quantum yield ($\Phi$, Equation S3.3) is dependent on the concentration of pyridine.

$$\Phi = 0.44 \text{[Py]} + 2.8 \times 10^{-4}$$  \hspace{1cm} (S3.3)

We followed the actinometer reaction under the different POM and DOM sensitizers condition. In addition we monitored the reaction in the presence of uncoated SiO$_2$ beads of both sizes. The measured rates are reported in Table S3.3. The quantum yield function is given in Equation S3.4.

$$k_{p_{N-Py}} = 2.303 \times E_\lambda \times \varepsilon_\lambda \Phi$$ \hspace{1cm} (S3.4)

$k_{p_{N-Py}}$ is the observed rate, $\varepsilon_\lambda$ is the molar extinction coefficient ($6.3 \times 10^3$) and $E_\lambda$ is the irradiance. Using Equation S3.4, and the known value of $\Phi$ from Equation S2, the irradiance is estimated.
Table S3.3. Irradiance of the solutions in the presence of different sensitizers

<table>
<thead>
<tr>
<th>Solution</th>
<th>$k_{\text{PN-Py}} \times 10^{-4} \text{M}^{-1} \text{s}^{-1}$</th>
<th>$I \times 10^{-6} \text{W m}^{-2}$</th>
<th>$I_{\text{n}}/I_{\text{blank}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOM</td>
<td>0.73</td>
<td>1.21</td>
<td>0.55</td>
</tr>
<tr>
<td>POM-1</td>
<td>1.00</td>
<td>1.64</td>
<td>0.75</td>
</tr>
<tr>
<td>POM-0.5</td>
<td>1.18</td>
<td>1.94</td>
<td>0.88</td>
</tr>
<tr>
<td>Blank</td>
<td>1.34</td>
<td>2.20</td>
<td>1.00</td>
</tr>
<tr>
<td>SiO$_2$-1</td>
<td>1.46</td>
<td>2.40</td>
<td>1.09</td>
</tr>
<tr>
<td>SiO$_2$-0.5</td>
<td>1.65</td>
<td>2.71</td>
<td>1.23</td>
</tr>
</tbody>
</table>

The results reported in Table S3.3 suggest that the screening is partially compensated by the scattering of the particles. In the solution containing POM-0.5 and in the one containing POM-1.0 the OM content is the same resulting in a larger number of particles in solution in the case of POM-0.5 (~$1.09 \times 10^{13}$ units/L) compared to POM-1.0 (~$3.25 \times 10^{12}$ units/L). This can explain the larger enhancement due to scettering effect in the case of POM-0.5 compared to POM-1.0 particles, both coated and uncoated.
FFA consumption in heterogeneous solution

In order to exclude the geometric effect explanation for the difference in the \([^{1}O_{2}]_{aq}^{DOM}\) and \([^{1}O_{2}]_{aq}^{OM}\) detected by FFA, we estimated the difference of FFA inside and outside DOM under the experimental condition. To do so, we need to compare the moles of FFA consumed per second in the OM unit with the moles of FFA per second supplied to the OM region through diffusion. We calculate the gradient between inside the DOM and outside the DOM as a limiting case, since we know the \([^{1}O_{2}]_{OM}\) from the TPMA measurement.

The first step is to estimate the consumption rate of FFA inside one DOM aggregate \(\left(\frac{dn_{FFAOM}}{dt}\right)\) (Equation S3.5)

\[
\frac{dn_{FFAOM}}{dt} = k_{rxn}[FFA][^{1}O_{2}]_{OM} \times \frac{4}{3} \pi r^3
\] 

(S3.5)

Where \(k_{rxn}\) is the FFA and \(^{1}O_{2}\) bimolecular reaction rate constant (estimated to be \(8.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\)), \([FFA]\) is the concentration of FFA in solution (\(10^{-4} \text{ M}\)), \([^{1}O_{2}]_{OM}\) is the concentration of \(^{1}O_{2}\) in the organic matter (estimated by TPMA to be roughly \(3 \times 10^{-11} \text{ M}\), Table 3.1) and \(r\) is the radius of the OM aggregate (that, as a simplification, we assume spherical) and is estimated to be \(1 \text{ nm}\).

Equation 3.6 represents the moles of FFA supplied to the OM through the OM surface area and is equal to the flux (derived from Fick’s law) times the surface area (Equation S3.6).

\[
J \times SA = D \times \frac{[FFA]_{a} - [FFA]_{l}}{l} \times \frac{4}{3} \pi r^2
\] 

(S3.6)

Where \(J\) is the diffusion flux in units of mmol s\(^{-1}\) cm\(^{-2}\), \(SA\) is the surface area of the OM aggregate (cm\(^2\) with radius \(r\)), \(D\) is the diffusivity (estimated, according to the Stoke-Einstein equation,\(^{7}\) to be \(4.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}\) for FFA) and \(l\) is gradient length.
The maximum gradient length would be through the entire radius of the OM particle, in which case \( l = r \).

Combining Equation S3.5 and S3.6 we obtain Equation S3.7

\[
k_{rxn}[\text{FFA}][^{1}\text{O}_2]_{OM} \times \frac{4}{3} \pi r^3 = D \times \frac{[\text{FFA}]_0-[\text{FFA}]}{r} \times 4\pi r^2 \tag{S3.7}
\]

And upon rearrangement we obtain Equation S3.8

\[
k_{rxn}[\text{FFA}][^{1}\text{O}_2]_{OM} \times \frac{1}{3} r^2 = D \times ([\text{FFA}]_0 - [\text{FFA}])_r \tag{S3.8}
\]

Solving Equation 3.8 for \(([[\text{FFA}]_0-\text{[FFA]}])_r\) we obtain a difference between outside and inside OM of 1.8 x 10^{-16} M (corresponding to 0.00000000001 % change). We can conclude that under the experimental condition we do not have a significant gradient from the inside to the outside of the OM region.

If we set \(([[\text{FFA}]_0-\text{[FFA]}])_r\) to a significant change such as 10 % (10^{-5} M), using the same relationship, we can propose the scenarios where such an eventuality could occur.

One possibility is to have 10^{11} higher concentrations of \(^1\text{O}_2\) in OM. This would mean a \([^{1}\text{O}_2]\) of 1 M, which is physically impossible because it exceed of several order of magnitude the solubility of oxygen in water.

A second possibility would be to have a much larger OM aggregate. In order to obtain a 10^{-5} M difference between the interior and exterior concentrations of \(^1\text{O}_2\), we need a radius of OM of 100 \(\mu\)m.

A more intuitive explanation of this problem is given by calculating the distance FFA can diffuse in the time it takes to consume 10% of it through the reaction with \(^1\text{O}_2\). The calculated time for 3 x 10^{-11} M \(^1\text{O}_2\) is 120 s, which corresponds to a diffusion distance of 230 \(\mu\)m. For distances significantly shorter than 230 \(\mu\)m, diffusion is much faster than loss of FFA.
References


Chapter 4

On the use of hydroxyl radical kinetics to assess the number average molecular weight of dissolved organic matter

Elena Appiani, Sarah E. Page and Kristopher McNeill

Published in

Environmental Science & Technology 2014, 48, 11794-11802.
4.1 Table of content (TOC Art)

4.2 Abstract

Dissolved organic matter (DOM) is involved in numerous environmental processes, and its molecular size is believed to be important in many of these processes, such as DOM bioavailability, DOM sorptive capacity, and the formation of disinfection by-products during water treatment. The size and size distribution of the molecules composing DOM remain an open question. In this contribution, an indirect method to assess the average size of DOM is described, which is based on the reaction of hydroxyl radical (HO•) quenching by DOM. HO• is believed to be relatively unselective, reacting with nearly all organic molecules with similar rate constants. Literature values for HO• reaction with organic molecules were surveyed to assess the unselectivity of DOM and to determine a representative quenching rate constant ($k_{rep}=5.6 \times 10^9 \text{ M}^{-1} \text{s}^{-1}$). This value was used to assess the average molecular weight of various humic and fulvic acid isolates as model DOM, using literature HO• quenching constants, $k_{C,DOM}$. The results obtained by this method were compared with previous estimates of average molecular weight. The average molecular weight ($M_n$) values
obtained with this approach are lower than the $M_n$ measured by other techniques such as size exclusion chromatography (SEC), vapor pressure osmometry (VPO), and flow field fractionation (FFF). This suggests that DOM is an especially good quencher for HO•, reacting at rates close to the diffusion-control limit. It was further observed that humic acids generally react faster than fulvic acids. The high reactivity of humic acids toward HO• is in line with the antioxidant properties of DOM. The benefit of this method is that it provides a firm upper bound on the average molecular weight of DOM, based on the kinetic limits of the HO• reaction. The results indicate low average molecular weight values, which is most consistent with the recent understanding of DOM. A possible DOM size distribution is discussed to reconcile the small nature of DOM with the large-molecule behavior observed in other studies.

4.3 Introduction

Dissolved organic matter (DOM) is ubiquitous and is directly involved in numerous important processes in environmental aquatic chemistry. For instance, DOM has been shown to play roles in redox reactions,¹ fate and transport of pollutants,² nutrient cycling in aqueous systems,³ and microbial respiration.⁴ Decades of research has led to the currently accepted model that describes DOM as being composed of a complex mixture of molecules of different size, structures, and chemical properties dynamically associated and stabilized by non-covalent interactions.⁵,⁶ However, DOM molecular size and size distribution are a subject of ongoing research.

The average molecular weight and size of DOM is often mentioned as an important factor influencing its bioavailability.⁷ DOM molecular size affects the sorptive capacity and strength of binding with organic compounds.⁸,⁹
evidence that DOM can differently affect the sorption capacity of the materials it is bound to, such as carbon nanotubes, depending on its molecular weight. Similarly, formation of water disinfection by-products has been shown to depend on DOM size.

The definition of size for mixtures is not trivial. This is well known in polymer chemistry where two different molecular weight averages, the number average ($M_n$) and the weighted average ($M_w$) are widely employed depending on the property under investigation and the analytical technique in use. The number average, $M_n$, is the most intuitive descriptor, being the simple arithmetic average molecular mass of all of the components with each component weighted equally. However, many detection methods are weighted toward larger molecules and thus do not give $M_n$ directly. One of the best examples is dynamic light scattering (DLS) analysis, which preferentially detects larger molecules in a mixture, given the $6^{th}$ power dependence of scattering efficiency on radius. Measurements based on colligative properties, such as ebulliometry, which measures boiling point depression, are notable exceptions that count each molecule equally.

One possible method to measure the range of $M_n$ is based on the quenching of hydroxyl radical (HO•) by DOM, and this is the focus of the present work. HO• is often discussed as having very low selectivity, reacting with nearly all organic molecules at near-diffusion-controlled rates. This notion has been taken advantage of in the protein footprinting technique, which uses the high reactivity and low selectivity of HO• to determine solvent-exposed amino acids in folded proteins. If one makes the assumption that HO• reacts with all organic molecules unselectively, then the number of molecules in solution can be enumerated by assessing how well HO• is quenched in that solution. Knowing the number of molecules in solution and
their average molecular formula, in turn, would allow one to estimate the number average molecular weight ($M_n$) of the solutes.

The present work contains two components. First, a large collection of previously measured HO• rate constants was examined to assess the unselective reactivity assumption and to determine a representative rate constant to use for HO• reaction with organic molecules. Second, published rate constants for HO• quenching by humic substance isolates were used in combination with the representative rate constant to estimate $M_n$ of each isolate. In addition, we compared our results with previous estimates of DOM average molecular weight and discuss a molecular weight distribution model that reconciles the various results.

4.4 Approach and Methods

Estimation of a general HO• quenching rate constant

In order to use HO• quenching to determine the number of molecules in solution, the first goal was to determine a suitable quenching rate constant that could be used to describe the reaction of HO• with a DOM mixture. The concept of a general rate constant for HO• is not new and has been recently reported in a DOM study by Arakaki et al.\textsuperscript{16} In that study, a general scavenging rate constant for reaction of HO• was estimated using the same approach of compiling a training set.\textsuperscript{16}

Bimolecular rate constants of HO• reacting with numerous compounds are available because of the importance of HO• in fields such as biology, environmental sciences, and medicine. We compiled published rates of HO• reaction with organic compounds.\textsuperscript{17-22} Only organic molecules of $C_{c}H_{h}N_{n}O_{o}$ composition were taken into account because the elemental analysis of DOM reveals that C, H, N and O account for more than 98% of the mass of International Humic Substances Society (IHSS) isolates.\textsuperscript{23} We selected a large range of molecular sizes because DOM is
heterogeneous and both small organic molecules such as methanol\textsuperscript{24} and large protein-like biomolecules have been found in DOM.\textsuperscript{25} We also selected a large range of functional groups to reflect the structural diversity of DOM. The selected interval ranges from 1 C and 32 g mol\textsuperscript{-1} for methanol up to 156 C and 2018 g mol\textsuperscript{-1} for a fullerene-C\textsubscript{60}-\(\gamma\)-cyclodextrin complex.

A rate constant of \(5.6 \times 10^9\) M\textsuperscript{-1} s\textsuperscript{-1} was chosen as a representative rate constant (\(k_{\text{rep}}\)) for DOM based on the distribution of rate constants found in this data set (see the Results and Discussion section for more on the selection of this rate constant).

\textit{Estimation of number average molecular weight (\(M_n\))}

The \(M_n\) value of a DOM sample was estimated using four different \(k\) values (Table 4.1).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|l|}
\hline
\textbf{Rate} & \textbf{Unit} & \textbf{Description} \\
\hline
\(k_q\) & M\textsuperscript{-1} s\textsuperscript{-1} & Bimolecular rate constant values for HO\textbullet quenching by known molecules \\
\hline
\(k_{\text{rep}}\) & M\textsuperscript{-1} s\textsuperscript{-1} & Representative bimolecular rate constant for HO\textbullet quenching by organic molecules \\
\hline
\(k_{C,\text{DOM}}\) & L mol\textsuperscript{-1} s\textsuperscript{-1} & Bimolecular rate constant values for HO\textbullet quenching by DOM on a per molar-carbon basis \\
\hline
\(k_{\text{DOM}}\) & L g\textsubscript{DOM}\textsuperscript{-1} s\textsuperscript{-1} & Bimolecular rate constant values for HO\textbullet quenching by DOM on a per mass basis \\
\hline
\end{tabular}
\caption{Description of the \(k\) values used in the estimation of the \(M_n\) of DOM isolates.}
\end{table}

First, for known molecules, the HO\textbullet quenching bimolecular rate constants are reported as \(k_q\) in units of per molar per second (M\textsuperscript{-1} s\textsuperscript{-1}). Second, as mentioned in the previous section and discussed in the Results and Discussion section below, these \(k_q\) values were used to determine a representative bimolecular rate constant for HO\textbullet reaction with organic molecules, \(k_{\text{rep}} = 5.6 \times 10^9\) M\textsuperscript{-1} s\textsuperscript{-1}. Third, for DOM, which is composed of an unknown mixture of molecules, the rate constants for HO\textbullet quenching are commonly reported as \(k_{C,\text{DOM}}\), in units of liters per mole carbon per second (L
mol\textsuperscript{−1} s\textsuperscript{−1}). Fourth, for this paper, we have converted \(k_{C,\text{DOM}}\) to a per-mass rate constant of quenching (\(k_{\text{DOM}}\)), with units of liters per gram carbon per second (L g\textsubscript{\text{DOM}}\textsuperscript{−1} s\textsuperscript{−1}) \textit{(Equation 4.2, described below)}.

The number average molecular weight of a DOM sample was determined from the DOM-specific HO• quenching rate constant (\(k_{\text{DOM}}, \text{L g}_{\text{DOM}}\textsuperscript{−1} \text{s}^\text{−1}\)) and the general HO• rate constant (\(k_{\text{rep}}, \text{M}^\text{−1} \text{s}^\text{−1}\)). The DOM-specific rate constants have been determined for various DOM isolates\textsuperscript{26-34} In this study we selected rate constants of quenching of HO• by isolated DOM material, choosing only the studies that used IHSS standard isolates\textsuperscript{26-34} and non-standard materials where the elemental composition of the DOM was reported, since this information is a prerequisite for the calculation \textit{(Equation 4.2)}, although it is not always reported in HO• quenching studies.

The average molecular weight of DOM \((M_a)\) was obtained by dividing the representative rate constant \((k_{\text{rep}})\) by the DOM-specific HO• quenching rate constant \((k_{\text{DOM}})\) \textit{(Equation 4.1)}.

\[
M_a \left[ \frac{g_{\text{DOM}}}{\text{mol}_{\text{DOM}}} \right] = \frac{k_{\text{rep}} \left[ \text{mol}_{\text{DOM}}^{-1} \text{L} \text{s}^{-1} \right]}{k_{\text{DOM}} \left[ \frac{\text{g}_{\text{DOM}}}{\text{L}} \text{s}^{-1} \right]} \tag{4.1}
\]

As the rate constant of HO• quenching by DOM is usually reported on a carbon basis \((k_{C,\text{DOM}})\) the literature data was converted to the per-mass-based rate constant \((k_{\text{DOM}})\) using the carbon atomic mass \((MW_C)\) and the carbon fraction in DOM \((f_C)\) according to \textit{Equation 4.2}.

\[
k_{\text{DOM}} \left[ g_{\text{DOM}}^{-1} \text{L} \text{s}^{-1} \right] = k_{C,\text{DOM}} \left[ \text{mol}_{C}^{-1} \text{L} \text{s}^{-1} \right] \frac{1}{MW_C} \left[ \frac{\text{mol}_C}{\text{g}_C} \right] f_C \left[ \frac{g_C}{g_{\text{DOM}}} \right] \tag{4.2}
\]
The ratio of $k_{rep}$ and $k_{C,DOM}$ is the average number of carbons per DOM molecule (Equation 4.3).

$$\frac{k_{rep}}{k_{C,DOM}} \left( \frac{mol_{DOM}^{-1} Ls^{-1}}{mol_c^{-1} Ls^{-1}} \right) = \left( \frac{mol_c}{mol_{DOM}} \right)$$  (4.3)

Notice that with the method described above it is not possible to distinguish between aggregates and small molecules. This is important since many authors have suggested that DOM self-aggregates. Such aggregates would appear as “large molecules” to this method, and the true $M_n$ considering the building blocks of these aggregates as separate molecules would be smaller than found by this method.

4.5 Results and Discussion

*Determining the general HO• quenching rate constant*

To determine a representative rate constant for quenching of HO• by natural organic matter, we examined previously measured rate constants for a wide variety of organic molecules. Examining the rate constants\textsuperscript{17-22, 35-39} (Figure 4.1), we observed that the experimental data were distributed between $6 \times 10^7$ and $5 \times 10^{10}$ M\(^{-1}\)s\(^{-1}\). The histogram (Figure 4.1B) shows that most of the rate constants cluster in the middle of this range, with 75\% of the values being between $3 \times 10^9$ and $10 \times 10^9$ M\(^{-1}\)s\(^{-1}\). The distribution is also asymmetric, tailing toward lower rate constants, which might be expected given the fact that the diffusion-controlled limit for a bimolecular reaction in water is on the order of $10^{10}$ M\(^{-1}\)s\(^{-1}\). The very highest values in this compilation, which approach $5 \times 10^{10}$ M\(^{-1}\)s\(^{-1}\), are essentially at the diffusion-controlled limit.
Figure 4.1 Distribution of \( \text{HO}^\cdot \) quenching rate constants for organic molecules with formula \( \text{C}_c\text{H}_h\text{N}_n\text{O}_o \). A The dots correspond to published \( \text{HO}^\cdot \) quenching rate constants\(^{17-22,35-39} \) versus the molecular weight of the quenchers. Both axes are reported on a logarithmic scale. B A histogram of the rate constants from A. The colored lines in the histogram are possible representative \( \text{HO}^\cdot \) quenching bimolecular rate constants (\( k_{\text{rep}} \)) selected using different criteria: red line as the average of all values (\( 3.9 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \)); blue dotted line, average of all values excluding molecules less than 150 g/mol (\( 5.5 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \)); and, green dashed line as the mode of the distribution (\( 5.6 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \)). In this study we choose to use the value represented with the green dashed line, \( 5.6 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \).

The data presented in Figure 4.1 cover a wide range of rate constants and argue against the assumption of \( \text{HO}^\cdot \) being unselective in its reactivity. The outliers indicate that there are some structures that lead to exceptionally fast (e.g., electron-rich phenols) and exceptionally slow reactions with \( \text{HO}^\cdot \) (e.g. electron-poor molecules lacking aromatic substructures) (Table S4.1).

Happily, for considering the reactivity of DOM, the distribution of rate constants narrows considerably for larger molecules (>150 g/mol): 90% of the rate constants in this group are in the \( 1 - 30 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) range. This is possibly due to a balancing effect, in which larger molecules have both fast-reacting and slow-reacting substructures within the same molecule. It is also important to note that for the
method to work, one needs only the average rate constant for the entire DOM mixture. If the high and low outliers balance each other, then an accurate $M_n$ will be calculated. If the training set (i.e., data in Figure 4.1) is sufficiently similar to DOM, then it should not matter that the individual components have rate constants that span a wide range; the average should be close to what we have chosen. If, however, DOM contains a significant pool of either fast-reacting or slow-reacting molecules that shifts the average quenching rate constant away from our estimate of $k_{rep}$, then inaccurate $M_n$ values will be obtained. This possibility is discussed in more detail in the next section.

A general rate constant for HO• reaction with DOM was estimated in different ways: (1) Assuming that the suite of molecules taken into account is representative of DOM and averaging the rate constants of all the molecules considered above. This approach resulted in a general HO• quenching rate constant of $3.9 \times 10^9$ M$^{-1}$ s$^{-1}$ (red line Figure 4.1B) (2) Averaging all the rate constants of quenching by molecules with a molecular weight higher than 150 g/mol, because it is generally believed that low molecular weight material (<150 g/mol) is not the major constituent of DOM. This approach resulted in a general rate constant of $5.5 \times 10^9$ M$^{-1}$ s$^{-1}$ (blue dotted line Figure 4.1B). (3) Selecting the mode of the distribution. This approach resulted in a value of $5.6 \times 10^9$ M$^{-1}$ s$^{-1}$ (green dashed line Figure 4.1B). The HO• quenching rate constant obtained with the three approaches are comparable. We choose to use the mode approach in the subsequent calculations, giving $5.6 \times 10^9$ M$^{-1}$ s$^{-1}$ as the general HO• quenching rate constant, in part because we felt that the training set was overrepresented in slow-reacting N-rich molecules. Note that choosing a lower rate constant would result in lower calculated $M_n$ values (see Equation 4.1). The general rate constant previously reported by Arakaki et al, $6.4 \times 10^9$ M$^{-1}$ s$^{-1}$, is the average of
the rate constants reported in their training set, and it is in good agreement with our estimate.\textsuperscript{16} Arakaki et al analyzed a very large dataset (1400 individual molecules) that did not exclude any organic compounds. We attribute the difference between Arakaki’s $k_{\text{rep}}$ and ours to our exclusion of fast-reacting S- and P-containing compounds in our dataset.

**Determination of DOM size by HO• reaction**

Numerous groups have determined rate constants for the quenching of HO• by DOM in the last 15 years.\textsuperscript{26-34} By far the best studied of these is Suwannee River Fulvic Acid I (SRFA I),\textsuperscript{26-33} for which at least ten different rate constant determinations by different methods have been reported (Table 4.2). It is instructive that even for SRFA I, the best studied isolate, the $k_{C,\text{DOM}}$ value is not known to a high level of precision. This is important since the near factor-of-four spread between the lowest and highest $k_{C,\text{DOM}}$ values leads directly to a near factor-of-four range in the calculated $M_n$ values for SRFA I. The accuracy of $M_n$ values obtained from HO• quenching kinetics is limited to the accuracy of $k_{C,\text{DOM}}$ values. Nevertheless, even with large uncertainties, the $M_n$ estimates by this approach remain useful.

Using the rate constants of HO• quenching by DOM in Tables 4.1 and 4.2, the representative HO• quenching rate constant ($k_{\text{rep}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), and Equations 4.1-4.3, we calculated $M_n$ and the average number of carbon atoms per DOM molecule, as summarized in Table 4.2 for IHSS isolates (~100-1000 Da and 5-47 C) and Table 4.3 for other isolates (276-490 Da and 12-21 C).

Analyzing the results obtained with this method, we observe two unexpected results. First, in general, the $M_n$ values obtained using this method are quite low (Table 4.2 and Table 4.3) compared to the values published in the literature obtained by various analytical techniques (Appendix C, Tables S4.2-S4.3). Second, the humic
acid samples appear to have lower $M_n$ values than fulvic acid samples, which does not reflect the trends reported in previous studies (Appendix C, Table S4.2).

With regard to the first observation, one must consider what changes would yield a higher $M_n$ value. The number average molecular weight, $M_n$ is determined by $k_{rep}$ and $k_{C,DOM}$ (Equation 4.1), thus inaccuracies in those two values will affect the quality of $M_n$. An overestimation of $k_{C,DOM}$ will result in an underestimation of $M_n$ by the same factor, since $M_n$ and $k_{rep}$ are inversely proportional (Equation 4.1). On the other hand, increasing $k_{rep}$ will increase $M_n$ by the same factor (Equation 4.1). Inaccuracies in $k_{rep}$ and $k_{C,DOM}$ may be contributing to the lower than expected values of $M_n$ reported in Table 4.2. There are two possibilities: Either $k_{rep}$ is underestimated or $k_{C,DOM}$ is overestimated. If the true value of $k_{rep}$ is higher than our estimate, it could not be much higher, because our estimate of $5.6 \times 10^9$ M$^{-1}$ s$^{-1}$ is already close to the diffusion-controlled limit. However, analyzing Table S4.1 we notice that the exceptionally fast quenching reaction rate constants are reported for electron-rich phenols, a moiety known to be a DOM constituent. This suggests that the rate constant chosen might underestimate the overall reactivity of DOM, since it does not take into account these highly reactive species. If we double $k_{rep}$ to $1.1 \times 10^{10}$ M$^{-1}$ s$^{-1}$, for example, the $M_n$ range would change from $\sim$120-1000 Da (Table 4.2) to $\sim$220-1800 Da. Thus, focusing only on the fulvic acid results, the $M_n$ calculated with the higher $k_{rep}$ (PLFA 680 g/mol, SRFA I 1220 g/mol, and SRFA II 1520 g/mol) would match the values reported by other techniques such as VPO, FFF, and SEC (Appendix C, Table S4.2). If an incorrectly chosen $k_{rep}$ value is the reason for the low $M_n$ values, this suggests that the fulvic acid ensembles react with HO• about two times faster than the mixture of molecules chosen as the training set (i.e., $k_{rep,fulvic} \sim 1.1 \times 10^{10}$ M$^{-1}$ s$^{-1}$).
The second possibility is that the $k_{C,DOM}$ values are artificially high. It is important to note that $k_{C,DOM}$ data reported in Table 4.2 are from different studies, performed in different laboratories, and using different techniques. There is no clear trend in the scavenging rate constants with HO• generation methods, which is possibly complicated by variations in both HO• generation techniques and HO• detection methods. For example, in the case of SRFA I, HO• generation by both nitrate photolysis and ozonation results in HO• scavenging rates that vary by a factor of two in different studies (Table 4.2). There is previous evidence that benzoic acid, a representative aromatic probe for HO•, responds differently to standard HO• production methods, which may be a contributing factor to the variability of HO• scavenging rates observed in these studies. The unknown nature of this variability does make comparison of results between laboratories and different techniques challenging, but the data in Table 4.2, when taken together, should reflect the order of magnitude of the $M_n$ of the isolates. The focus of this work is to explore what HO• quenching kinetics say about the $M_n$ of DOM rather than assessing the quality of the data reported in these studies. Thus, all of the compiled HO• scavenging data (Table 4.2) have been treated equally. When examining the data as a whole, there is no indication that these previous studies have produced $k_{C,DOM}$ values that are artificially high.

The second unexpected result, that humic acids appear smaller than fulvic acids, is also connected to both the choice of $k_{rep}$ and the heterogeneity of the source of the data reported in Table 4.2. In particular, in essentially every study of humic and fulvic acids, it has been shown that humic acids have a larger average molecular size than fulvic acids (Appendix C, Table S4.2). Looking more closely at the data in Table 4.2, there are four studies that examined the HO• quenching of both SRFA and
SRHA, all using different methods.\textsuperscript{29-32} In three of the four cases, the $k_{C,DOM}$ values for SRHA were higher than those for SRFA (by a factor of 2.2 on average). If we take as a given that SRHA has a higher average $M_n$ than SRFA, according to Equation 4.1, the only way $k_{DOM}$ can be higher for SRHA than SRFA is if the value for $k_{rep}$ is also higher for SRHA than SRFA, but by an even greater factor. Assuming, the $M_n$ value for SRHA is about 50\% larger than for SRFA (see Table S2), $k_{rep, humic}$ would be about 3.3 times $k_{rep, fulvic}$ or approximately $3.4 \times 10^{10}$ M$^{-1}$ s$^{-1}$. This is consistent with the fact that SRHA and SRFA have different chemical compositions, with SRHA having higher aromatic and phenolic content. Table S4.1 (Appendix C) shows that electron-rich aromatic compounds such as phenols can have an exceptionally high reactivity towards HO•.

Westerhoff, et al. have collected a dataset (Table 4.3) that supports the suggestion that the $k_{rep}$ value of $5.6 \times 10^9$ M$^{-1}$ s$^{-1}$ is too low.\textsuperscript{29} In the Westerhoff et al. study, HO• quenching rate constants were obtained for a range of organic matter isolates from a variety of natural water sources. The isolation procedure (XAD8 sorption at pH 2 with elution at pH 13) presumably resulted in a mixture of humic and fulvic acids, but the characterization data suggests that these isolates are dominated by fulvic acid character. For four of the source waters, $M_n$ values from VPO data are also available.\textsuperscript{41} Table 4.3 lists the $M_n$ values from HO• quenching calculated using a $k_{rep}$ value of $5.6 \times 10^9$ M$^{-1}$ s$^{-1}$ as well as a two-times higher value of $1.1 \times 10^{10}$ M$^{-1}$ s$^{-1}$, as suggested by the fulvic acid data discussed above. The $M_n$ values calculated using the low $k_{rep}$ value are very low and not in agreement with the VPO data. On the other hand, the $M_n$ values calculated using the higher $k_{rep}$ value are in good agreement with the VPO data.
The data in **Table 4.3** also reinforce the postulate that the HO• quenching rate constant varies with the composition of the organic matter sample, with higher aromatic and phenolic content giving rise to higher rate constants. The aromatic carbon content was estimated for the samples examined by Westerhoff, et al. (**Table 4.3**), and a correlation between $k_{C,DOM}$ and % aromatic C content is observed ($r^2 = 0.60$; **Figure S4.1**). From **Equation 4.1**, there are only two ways for more aromatic-rich samples to have higher per-mole-carbon rate constants of HO• quenching. Either the more aromatic-rich samples have lower average molecular weights or they have higher intrinsic reactivity toward HO•. We believe the latter explanation is more compelling, especially given that aromatic compounds are known to react with HO• with especially high rate constants.$^{42}$
Table 4.2. Hypothetical number average molecular weight ($M_n$) values and average number of carbon atoms per DOM unit calculated for International Humic Substance Society (IHSS) DOM isolates based on uniform reactivity towards HO• ($k_{rep} = 5.6 \times 10^{9} \text{M}^{-1} \text{s}^{-1}$). The HO• production and detection methods are also listed for each entry.

<table>
<thead>
<tr>
<th>Organic matter source</th>
<th>%C</th>
<th>$k_{C,DOM}$ ((10^8 \text{M}^{-1} \text{C}^{-1} \text{s}^{-1}))</th>
<th>HO• production method</th>
<th>HO• detection method</th>
<th>Reference</th>
<th>Average number of C per unit DOM</th>
<th>$M_n$ g mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suwanee River Fulvic Acid I</td>
<td>52.44</td>
<td>1.4</td>
<td>Nitrate photolysis</td>
<td>Nitrobenzene</td>
<td>26</td>
<td>40</td>
<td>915</td>
</tr>
<tr>
<td></td>
<td>1.4 (±0.16)</td>
<td></td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>28</td>
<td>40</td>
<td>915</td>
</tr>
<tr>
<td></td>
<td>1.6 (±0.04)</td>
<td></td>
<td>Radiolysis</td>
<td>SCN• competition</td>
<td>28</td>
<td>35</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td></td>
<td>Radiolysis</td>
<td>SCN• competition</td>
<td>27</td>
<td>35</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>1.9 (±0.07)</td>
<td></td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>28</td>
<td>29</td>
<td>674</td>
</tr>
<tr>
<td></td>
<td>2.1 (±0.18)</td>
<td></td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>30</td>
<td>27</td>
<td>610</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td></td>
<td>Radiolysis</td>
<td>14C-formate</td>
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<td>17</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td></td>
<td>Ozonation</td>
<td>p-Cl benzoic acid</td>
<td>29</td>
<td>15</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td></td>
<td>Nitrate photolysis</td>
<td>1-Cl-butane</td>
<td>32</td>
<td>15</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>5.3 (±0.1)</td>
<td></td>
<td>Fenton reaction</td>
<td>14C-formate</td>
<td>33</td>
<td>11</td>
<td>241</td>
</tr>
<tr>
<td><strong>Average (n = 10)</strong></td>
<td><strong>2.6 (±1.3)</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>26 (±11)</strong></td>
<td><strong>610 (±250)</strong></td>
<td></td>
</tr>
<tr>
<td>Suwanee River Fulvic Acid II</td>
<td>52.34</td>
<td>1.3 (±0.1)</td>
<td>Radiolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>43</td>
<td>988</td>
</tr>
<tr>
<td></td>
<td>2.4 (±0.5)</td>
<td></td>
<td>Photolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>23</td>
<td>535</td>
</tr>
<tr>
<td><strong>Average (n = 2)</strong></td>
<td><strong>1.9 (±0.8)</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>33 (±14)</strong></td>
<td><strong>760 (±320)</strong></td>
<td></td>
</tr>
<tr>
<td>Organic matter source</td>
<td>%C</td>
<td>$k_{C,DOM}$ (10^8 M^{-1}s^{-1})</td>
<td>HO• production method</td>
<td>HO• detection method</td>
<td>Reference</td>
<td>Average number of C per unit DOM</td>
<td>$M_0$ g mol^{-1}</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------</td>
<td>-------------------------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>---------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Suwanee River Fulvic Acid II</td>
<td>52.34</td>
<td>1.3 (±0.1)</td>
<td>Radiolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>43</td>
<td>988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4 (±0.5)</td>
<td>Photolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>23</td>
<td>535</td>
</tr>
<tr>
<td>Average (n = 2)</td>
<td>1.9 (±0.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33 (±14)</td>
<td>760 (±320)</td>
</tr>
<tr>
<td>Suwanee River Humic Acid I</td>
<td>52.55</td>
<td>2.3</td>
<td>Radiolysis</td>
<td>$^{14}$C-formate</td>
<td>31</td>
<td>24</td>
<td>556</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8</td>
<td>Nitrate photolysis</td>
<td>1-Cl-butane</td>
<td>32</td>
<td>8</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.1</td>
<td>Ozonation</td>
<td>$p$-Cl benzoic acid</td>
<td>29</td>
<td>7</td>
<td>158</td>
</tr>
<tr>
<td>Average (n = 3)</td>
<td>5.7 (±3.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13 (±10)</td>
<td>300 (±220)</td>
</tr>
<tr>
<td>Suwanee River Humic Acid II</td>
<td>52.63</td>
<td>10.36 (±0.02)</td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>30</td>
<td>5</td>
<td>123</td>
</tr>
<tr>
<td>Pony Lake Fulvic Acid</td>
<td>52.47</td>
<td>2.4 (±0.1)</td>
<td>Radiolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>23</td>
<td>534</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 (±0.6)</td>
<td>Photolysis</td>
<td>Terephthalate</td>
<td>34</td>
<td>13</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.9 (±0.8)</td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>30</td>
<td>8</td>
<td>186</td>
</tr>
<tr>
<td>Average (n = 3)</td>
<td>4.6 (±2.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15 (±7.6)</td>
<td>340 (±120)</td>
</tr>
<tr>
<td>Elliot Soil Humic Acid</td>
<td>58.13</td>
<td>1.2 (±0.2)</td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>30</td>
<td>47</td>
<td>963</td>
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<tr>
<td>Leonardite Humic Acid</td>
<td>63.81</td>
<td>6.5 (±0.3)</td>
<td>Radiolysis</td>
<td>Transient absorption</td>
<td>30</td>
<td>9</td>
<td>162</td>
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</table>
Table 4.3. Hypothetical number average molecular weight ($M_n$) values and average number of carbon atoms per DOM unit for various isolates calculated using the assumption of a uniform reactivity towards HO$^\cdot$ quenching rate constant. $M_n$ is estimated using two different $k_{rep}$ values. Quenching rate constant ($k_{C,DOM}$), percent carbon (%C) values, $M_n$ from VPO measurements and aromaticity are also listed.$^a\hspace{1mm}^b\hspace{1mm}^c$

<table>
<thead>
<tr>
<th>Organic matter source</th>
<th>%C</th>
<th>$k_{C,DOM}$ (10$^8$ M$^-1$s$^-1$)</th>
<th>$M_n$ g/mol for $k_{rep}$ $5.6 \times 10^9$ M$^-1$s$^-1$</th>
<th>$M_n$ g/mol for $k_{rep}$ $1.1 \times 10^{10}$ M$^-1$s$^-1$</th>
<th>$M_n$ from VPO $^4\hspace{1mm}^1$</th>
<th>Aromatic$^9$ (%C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater sample, MN</td>
<td>52.7</td>
<td>2.6</td>
<td>490</td>
<td>980</td>
<td>NA</td>
<td>11.9</td>
</tr>
<tr>
<td>Lake Michigan, IL</td>
<td>48.5</td>
<td>3</td>
<td>463</td>
<td>926</td>
<td>NA</td>
<td>14</td>
</tr>
<tr>
<td>Shingobee River, MN (1992)</td>
<td>51.1</td>
<td>3.3</td>
<td>399</td>
<td>798</td>
<td>NA</td>
<td>20.4</td>
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<tr>
<td>Lake Shingobee, MN</td>
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<td>3.4</td>
<td>387</td>
<td>774</td>
<td>NA</td>
<td>18.5</td>
</tr>
<tr>
<td>Lake Fryxell, Antarctica</td>
<td>55</td>
<td>3.5</td>
<td>408</td>
<td>816</td>
<td>NA</td>
<td>13.1</td>
</tr>
<tr>
<td>Missouri River, IA</td>
<td>55.4</td>
<td>3.5</td>
<td>405</td>
<td>810</td>
<td>640</td>
<td>20.4</td>
</tr>
<tr>
<td>Williams Lake, MN</td>
<td>51.8</td>
<td>3.5</td>
<td>371</td>
<td>742</td>
<td>NA</td>
<td>10.4</td>
</tr>
<tr>
<td>Yakima River at Kiona, WA</td>
<td>56.1</td>
<td>3.6</td>
<td>334</td>
<td>668</td>
<td>NA</td>
<td>25.3</td>
</tr>
<tr>
<td>Calif. State Project water, CA</td>
<td>48.1</td>
<td>4</td>
<td>350</td>
<td>700</td>
<td>NA</td>
<td>25</td>
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<tr>
<td>Yakima River at CleElum, WA</td>
<td>57.2</td>
<td>4</td>
<td>294</td>
<td>588</td>
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<td>26.6</td>
</tr>
<tr>
<td>Ohio River, OH</td>
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<td>4.1</td>
<td>296</td>
<td>592</td>
<td>606$^d$</td>
<td>24.3</td>
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<tr>
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<td>4.3</td>
<td>295</td>
<td>590</td>
<td>743</td>
<td>27.4</td>
</tr>
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<td>Ogeechee River, GA</td>
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<td>4.5</td>
<td>276</td>
<td>552</td>
<td>714</td>
<td>24.8</td>
</tr>
<tr>
<td>Shingobee River, MN (1993)</td>
<td>50.6</td>
<td>4.5</td>
<td>294</td>
<td>588</td>
<td>NA</td>
<td>24.5</td>
</tr>
</tbody>
</table>

$^a$ All the materials come from the same study and were isolated with the same method. $^b$ Data from Westerhoff et al.$^9$. $^c$ HO$^\cdot$ production method is ozonation, with p-Cl benzoic acid as HO$^\cdot$ probe $^d$ Average of spring and fall samples.
Assessment of using HO• quenching to determine molecular weight

The current study is focused on two main questions: First, is HO• unselective enough to assess a representative rate of quenching for a mixture? Second, can HO• quenching kinetics be used to estimate DOM $M_n$? With regard to the first question, characterizing HO• as unselective is not valid given that the pool of $k_q$ values analyzed in this study spans nearly three orders of magnitude ($6 \times 10^7$ M$^{-1}$ s$^{-1}$ – $4.8 \times 10^{10}$ M$^{-1}$ s$^{-1}$, Figure 4.1 and Appendix C Table S4.1). Since DOM is a mixture, we tested the idea of using a rate constant representative of the averaged rate constant for all DOM components. This rate constant was obtained by analyzing a training set of literature HO• quenching rate constants ($k_q$) (Figure 4.1) from molecules of CHNO composition. We observed that the $k_{rep}$ value obtained from this artificial mixture was likely too low to represent DOM and that the actual rate constants were probably two times higher for fulvic acids. Furthermore, humic acids were concluded to react even faster than fulvic acids (ca. 3 times faster). These observations suggest that fulvic acids and humic acids are much more reactive toward HO• than “average” organic molecules, in line with previous observations of DOM acting as an antioxidant.\(^{43}\)

With regard to the second question, the inability to determine a general average quenching rate constant for DOM means that $M_n$ values cannot be obtained precisely from HO• kinetics. Nevertheless, since DOM apparently reacts with HO• at close to diffusion-controlled rates, we can estimate a firm upper bound to DOM $M_n$. If we were to estimate the highest possible $M_n$, the prerequisite to the estimation are the lowest reported carbon content (48%, Table 4.3), the lowest reported $k_{C,DOM}$ ($1.2 \times 10^8$ M$_C^{-1}$ s$^{-1}$) and the extremely high average quenching rate constant estimated for humic acids ($3.4 \times 10^{10}$ M$^{-1}$
s$^{-1}$). Using these parameters we obtain 7000 Da as the upper limit for DOM $M_n$ value. Notice that the 7000 Da upper limit is calculated assuming that all the parameters synergistically contribute to the high molecular weight, namely the material must have a high intrinsic reactivity towards HO• (i.e. high aromatic content), the %C must be lower than average, and the reported $k_{C,DOM}$ must be below average. This coincidence has not been observed in the selection of studies reported (Table 4.2, Table 4.3, Appendix C, Table S4.4).

Similarly, this study provides guidelines to predict whether measured $k_{DOM}$ values are plausible. If we set a reasonable lower limit for FA and HA $M_n$ (600 Da and 900 Da) and we assume our estimates of $k_{rep, fulvic}$ and $k_{rep, humic}$ are accurate ($1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and $3.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$), we calculate the maximum $k_{C,DOM}$ values to be approximately $4 \times 10^8 \text{ M}_C^{-1} \text{ s}^{-1}$ and $9 \times 10^8 \text{ M}_C^{-1} \text{ s}^{-1}$, which correspond to $k_{DOM}$ values of approximately $4 \times 10^7 \text{ L g}_{DOM}^{-1} \text{ s}^{-1}$ and $7 \times 10^7 \text{ L g}_{DOM}^{-1} \text{ s}^{-1}$. Thus, measured values of $k_{C,DOM}$ exceeding this limit are either incorrect, or indicate the presence of additional HO• quenchers in the system.

The distribution of DOM sizes and its effect on $M_n$ determination

The disparate values for the size of DOM isolates, obtained from different experiments, is initially puzzling. For example, DOM is well-known to sorb relatively large organic molecules (e.g., benzo[α]pyrene (20 C atoms)\textsuperscript{44} and DDT (14 C atoms)\textsuperscript{45}), suggesting that DOM is much larger. On the other hand, $M_n$ values determined by VPO, and now by HO• quenching, indicate average molecular weights below 1000 Da. In addition, recent ultra-high resolution mass spectrometry studies demonstrate the presence of numerous low molecular weight species in DOM.\textsuperscript{46, 47} These apparently discordant results can be reconciled or rationalized by invoking a broad molecular weight
distribution. To illustrate this, consider the simple log-normal distribution model illustrated in Figure 4.2. There is precedent for using a log-normal distribution to describe the range of DOM molecular weights and to rationalize the size distribution of fulvic acids, because chromatograms obtained by HP-SEC analysis fit this kind of distribution.\textsuperscript{48} Such a distribution can be defined by Equation 4.4.\textsuperscript{48}

\[
f(x) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\left(\frac{1}{2\sigma^2}(\log(x) - \mu)^2\right)}
\]

(4.4)

The distribution is characterized by two coefficients, the mean value (\(\mu\)), that is the center of the distribution, and the standard deviation (\(\sigma\)), that represents the width of the distribution. In the distribution in Figure 4.2, we set \(\mu\) to 610 Da according to the average molecular weight calculated using the SRFA I data in Table 4.2, and \(\sigma\) to 0.316, according to the value reported by Cabannis et al. for SRFA I.\textsuperscript{48} In that study, the authors estimated the width of the distribution measuring the standard deviation of size exclusion chromatography results. This value of \(\sigma\), while arbitrary, does give good agreement with a prior study that showed 35% of SRFA passing through a nominal 500 Da filter.\textsuperscript{46} In this distribution, while half of the molecules have a mass of >700 Da (Figure 4.2, green dashed line), half of the total carbon is contained in molecules >800 Da (>34 C per molecule, 35% of the molecules, Figure 4.2, red line). Similarly, half of the scattering contribution would be given by molecules >900 Da (>38 C per molecule, 20% of the molecules, Figure 4.2, blue dotted line). This illustrates further that methods weighted by, for example, the carbon content of a molecule or its scattering properties, will yield discordant answers, and may explain why the same materials (e.g., SRFA I) have been assigned a wide range of average molecular weights.
Notice that this is only one possible distribution that could reconcile the apparent large and small aspects of DOM. Real distributions might be expected to be more complicated than a simple mono-modal distribution, as the sources of DOM are believed to be composed of large components, such as bio-macromolecules and bio-polymers, and low molecular weight components, such as secondary metabolites and membrane lipids.
Figure 2. One possible log-normal distribution of size of DOM versus molecules fraction. The black function represents the log normal distribution as percentage of molecules, obtained by Equation 4.4 setting $\mu$ (center of the distribution) equal to 610 Da and $\sigma$ to 0.316. The green dashed, red full and blue dotted curves represent the cumulative fraction of molecules, carbon and scattering response respectively. The dashed green function ($g(x)$) is the cumulative molecules fraction distribution and is described as $g(x) = \sum_{i=0}^{x} f(i)g(i) = \sum_{i=0}^{x} f(i)$, where $f(x)$ is the log normal distribution described above. The red full function ($h(x)$) is the cumulative carbon fraction distribution, and is described as $h(x) = \sum_{i=0}^{x} \frac{f(i)C_i}{\sum_{i=0}^{x} f(i)C_i}$ where $f(x)$ is the log normal distribution described above and $C_i$ is the number of carbons contained in the molecule of molecular weight $x$. The dotted blue function ($z(x)$) is the cumulative scattering contribution distribution, and is described as $z(x) = \frac{\sum_{i=0}^{x} f(i)r^6}{\sum_{i=0}^{x} f(i)r^6}$, where $f(x)$ is the log normal distribution described above and $r$ is the radius of the molecule of molecular weight $x$ estimated assuming a density of DOM of 1.05. The dashed green, full red, and dotted blue vertical line on the distribution, mark the fraction of small molecules needed to cover 50% of the molecules, of the carbon, and of the scattering response, respectively.
4.6 Environmental Implications

The HO• quenching rate constant of a DOM sample (k_{DOM}) is the ratio of the average rate constant for the molecules composing the DOM (k_{rep DOM}) and the number average molecular weight of the molecules composing the sample (M_n). In order to infer the value of one of the members of the ratio, information on the other are essential. In this study we calculated the M_n for DOM assuming its reactivity to be represented by the average reactivity of model organic molecules with HO•. The M_n values estimated with this method were found to be too low compared to previously reported M_n values. This is an indication that the k_{rep} value used was too low, meaning that DOM reactivity is at the higher end of the distribution of measured rate constants. Using reported values of DOM M_n, we estimated k_{rep} for fulvic and humic acids. The calculated value showed that DOM is highly reactive towards HO• and that specifically Suwanee River humic acid is approximately three times more reactive than Suwanee River fulvic acid. The high HO• scavenging ability of these materials is a further demonstration of the antioxidant properties of DOM.\(^{43}\)

In a system where HO• quenching rate constants are observed to vary over a series of samples (e.g., across a spatial gradient), it may be tempting to ascribe these changes to different M_n values in the samples. For example, HO• quenching rate constants have been seen to vary in in pristine Arctic surface waters\(^{49}\) and it may be reasonable to expect changes in the M_n values. Dong et al.\(^{50}\) analyzed the reactivity of different size fraction (<1 kDa to <10 kDa) of effluent organic matter with HO•. If each fraction has the same reactivity, then the M_n value would have to decrease by a factor of approximately 20 going from <1 kDa fraction to the <10 kDa fraction, which fits
perfectly with the rates measured by the authors.\textsuperscript{50} However, one cannot definitively state that the average molecular weight has changed between the samples without knowing the HO• reactivity of the material in each sample. The present study suggests that it is so far not possible to know \textit{a priori} the HO• quenching rate constant.

Despite the fact that it is not possible to unequivocally determine the size of DOM using HO• quenching rate constants, awareness of the directly proportional relationship between the two values allows one to put limits on reasonable values of both quenching rate constants for HO•, and DOM size. We estimated that the highest plausible quenching rate constant for fulvic acids and humic acids to be approximately $4 \times 10^8 \text{ M}_C^{-1} \text{s}^{-1}$ and $9 \times 10^8 \text{ M}_C^{-1} \text{s}^{-1}$, which correspond to values of approximately $4 \times 10^7 \text{ L g}_{\text{DOM}}^{-1} \text{s}^{-1}$ and $7 \times 10^7 \text{ L g}_{\text{DOM}}^{-1} \text{s}^{-1}$. For higher quenching rate constant values, the calculated $M_n$ for DOM would fall below 600 and 900 Da for fulvic acids and humic acids, respectively, even assuming an especially high reactivity. Similarly the study provides a firm upper limit to the $M_n$ for DOM. Assuming both an especially high intrinsic reactivity of DOM towards HO• and an especially slow measured rate of quenching, the maximum $M_n$ value that can be estimated is 7000 Da.

\section*{4.7 Acknowledgements}

We thank three anonymous reviewers for helpful comments that substantially improved the manuscript. We also gratefully acknowledge funding from the Swiss National Science Foundation (Grant no. CRSI22_127568).
4.8 References


14. Świętosławski, W., *Ebulliometric measurements*. 1945


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<thead>
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<th>Name</th>
<th>Structure</th>
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<th>MW (g/mol)</th>
<th>$k_q$ (M$^{-1}$ s$^{-1}$)</th>
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![Structures of various molecules](image-url)
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<th>Formula</th>
<th>MW (g/mol)</th>
<th>$k_q$ (M$^{-1}$ s$^{-1}$)</th>
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Table S4.2. Number averaged molecular weight ($M_n$) of DOM isolates from IHSS measured with different techniques.

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<th>$M_n$ (kDa)</th>
<th>Method</th>
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<td></td>
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<td>Technique</td>
<td>Property</td>
<td>Type of Technique</td>
<td>Advantages</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
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</tbody>
</table>
| SEC\textsuperscript{b} | Molecular size, detector used: UV | Fractionation | Respond directly to the volume | • UV detector depend on the molar extinction coefficient, which is hard to guess.\textsuperscript{19}  
• Doesn’t distinguish between single components or aggregates.  
• Sorption.  
• Electrostatic interaction.  
• Dependent on pre-calibration.\textsuperscript{20} | 1.4-3.9 | 9, 11-13, 15-18 |
| FFF\textsuperscript{c} | Diffusion coefficient | Fractionation | | • Diffusion coefficient | 1.15-3.73 | 10 |

\textsuperscript{a} Range of size of different International humic substances society isolates obtained using the specific technique. \textsuperscript{b} Size exclusion chromatography. \textsuperscript{c} Field flow fractionation.
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<tr>
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<td>Molar mass (distribution)</td>
<td>Fractionation</td>
<td>Offers info about distribution of MW</td>
<td>• Charge repulsion effects.</td>
<td>1-10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Solute adsorption.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Diffusion coefficient.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Swamping of charge effects.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Absorptivity varies with mass.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPO$^d$</td>
<td>Molar mass (distribution)</td>
<td>Colligative</td>
<td>It is not biased towards specific sizes because every specie counts 1</td>
<td>• Correction for ionizable compounds.</td>
<td>0.69-0.96</td>
<td>14</td>
</tr>
</tbody>
</table>

$^a$ Range of size of different International humic substances society isolates obtained using the specific technique. $^d$ Vapor pressure osmometry.
Table S4.4. Chemical composition of DOM isolates from IHSS. The data are reported Ref. 21.

<table>
<thead>
<tr>
<th>Material</th>
<th>Aromatic content (％)</th>
<th>Phenolic content (meq/g C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suwanee River Humic Acid I</td>
<td>37</td>
<td>4.24</td>
</tr>
<tr>
<td>Suwanee River Humic Acid II</td>
<td>31</td>
<td>3.72</td>
</tr>
<tr>
<td>Elliot Soil Humic Acid</td>
<td>50</td>
<td>1.87</td>
</tr>
<tr>
<td>Leonardite Humic Acid</td>
<td>58</td>
<td>2.31</td>
</tr>
<tr>
<td>Suwanee River Fulvic Acid I</td>
<td>24</td>
<td>2.91</td>
</tr>
<tr>
<td>Suwanee River Fulvic Acid II</td>
<td>22</td>
<td>2.84</td>
</tr>
<tr>
<td>Pony Lake Fulvic Acid</td>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure S4.1. Correlation between $k_{\text{C,DOM}}$ and % aromatic C. The data reported are from Westerhoff et al.\textsuperscript{22} The correlation suggests that the aromatic-rich samples have higher intrinsic reactivity toward HO•.
References


17. Perminova, I. V.; Grechishcheva, N. Y.; Petrosyan, V. S., Relationships between structure and binding affinity of humic substances for polycyclic aromatic


Chapter 5

Conclusions and outlook
5.1 Conclusions

The work presented in this thesis focused on different aspects of natural organic matter (NOM) photochemistry. The research theme that tied together the different studies is the interaction of NOM with photochemically produced reactive intermediates (PPRI) to elucidate NOM’s structure and reactivity. The first part of the thesis (chapters 2 and 3) focused on the photoproduction of singlet oxygen, $^1\text{O}_2$, and last part (chapter 4) focused on the correlation between quenching kinetics of hydroxyl radical, $\text{HO}^\cdot$, by NOM with the size of NOM.

The study on photochemically produced $^1\text{O}_2$ in the presence of dissolved organic matter (DOM) using furfuryl alcohol (FFA) and furfuryl esters had two main goals: To map out the reactivity of FFA in aqueous solution under different conditions, and to assess the effectiveness of alternate probes that contain the FFA core structure. With regard to the first goal, FFA’s reactivity with $^1\text{O}_2$ was found to be independent of the pH of the solution and to have a low dependence on temperature. In addition, the study verified that the reaction rate constant of FFA with $^1\text{O}_2$ is not affected by the substitution of D$_2$O for H$_2$O as a solvent. The similar reactivity under a wide variety of conditions makes FFA a versatile probe molecule for environmental studies.

The versatility of FFA further suggests that analogues could be useful as alternate probe molecules tuned to perform in distinct (micro)environments. For example, modification of FFA in the direction of more hydrophobic character could provide an FFA-based probe that preferentially partitions into DOM. The development of a set of modified FFA-based probe molecules to investigate the production of $^1\text{O}_2$ in natural waters aimed to demonstrate the possibility of designing DOM-bound probes molecules with the same mode of action as FFA. The use of a
structural series allowed us to verify a relationship between hydrophobicity of the probe molecules and the amount of $^1$O$_2$ detected. Our results showed that esterification of FFA had a low impact on the reactivity with $^1$O$_2$ and a correlation between the hydrophobicity of the probe molecules and the apparent $^1$O$_2$ detected was observed. However, finding a hydrophobic probe molecule that also partitions preferentially into DOM proved to be challenging as increased hydrophobicity also led to decreased solubility. These observations suggest that in addition to being highly hydrophobic, the probe molecule should contain structural features that prevents self-assembling without affecting its reactivity. TPMA, (2-[1-(3-tert-butyl(dimethyl)siloxy)phenyl]-1-methoxymethylene)adamantane), the probe successfully used in the literature to detect $^1$O$_2$ inside NOM, presents an adamantane group bound to the reactive site (Figure 5.1) that provide an almost spherical 3-dimensional structure. It is believed that this structure minimizes self-aggregation and allows low µM solubility of TPMA in water.

![Figure 5.1](image)

**Figure 5.1** Structure of TPMA, a $^1$O$_2$ probe molecule used for the detection of $^1$O$_2$ in the NOM microenvironment. The molecule’s 3-dimensional structure is hindered and the nearly spherical adamantane group minimizes self-aggregation.

The dual probe approach of combining a water soluble (FFA) and a hydrophobic (TPMA) probe in the study of NOM sensitized production of $^1$O$_2$ allowed us to investigate the photoactivity of particulate organic matter (POM), presented in chapter 3. Here, we demonstrated for the first time, that $^1$O$_2$ was
produced upon irradiation of engineered synthetic particles. These particles were prepared by layer-by-layer coating of a negatively charged silica support with a positively charged polymer and, subsequently, with a commercially available humic acid (negatively charged). The POM coating was stable under the experimental pH and ionic strength. Comparing the photosensitizing activity of the synthetic POM with the activity of a natural (suspended) sediment confirmed that the synthetic POM simulates well the behavior of (at least one type of) natural particles. We compared the $^1\text{O}_2$ oxygen generated upon irradiation of synthetic POM and DOM obtained by dissolving the same humic acid source used in the coating process. The POM internal concentration of $^1\text{O}_2$ detected by the hydrophobic probe molecule was similar or higher than the DOM internal concentration. These observations suggest that POM is a source of $^1\text{O}_2$ and is highly relevant in the degradation of sorbed species such as pollutant, viruses or microorganisms. However the evidence that the average concentration of $^1\text{O}_2$ in solution is nearly one order of magnitude lower for POM containing solution compared to DOM suggests that particles are not important for the $^1\text{O}_2$-mediated transformation of non-sorbed, freely dissolved species.

Finally, an indirect method to investigate the size of DOM based on its HO• quenching rate constants was analyzed (chapter 4). Based on the highly unselective reactivity of HO•, the HO• quenching rate constants by various organic molecules were surveyed to propose a general rate constant of quenching. Because this proposed rate constant was generated from a diverse mixture of organic molecules, we hypothesized that it was representative of the rate constant of HO• quenching of DOM. This general rate constant was used, combined with literature data of HO• quenching rate constants by various humic and fulvic acids isolates, to assess the average molecular weight of DOM materials. The results obtained were compared to
literature values for molecular weights estimates by different analytical techniques (such as size exclusion chromatography, flow field fractionation and vapor pressure osmometry). The comparison showed that our indirect method resulted in significantly lower molecular weight estimates. Furthermore, humic acids are generally known to be larger than fulvic acids, but results obtained with our proposed method show the opposite trend. These discrepancies suggest that our hypothesis that the general rate of quenching of HO• obtained from various organic molecules is representative of the rate constant of HO• quenching of DOM, must be rejected. Our observations rather suggest that DOM is an especially good quencher of HO•, reacting at rates close to the diffusion-controlled limit, and faster than at the proposed rate constant of low molecular weight organic molecules. The kinetic study of HO• quenching by DOM does not allow defining the molecular weight of DOM, but instead offers insights into the reactivity and structural properties of DOM.

5.2 Outlook

Our investigations demonstrate future research needs on the topics presented in this thesis.

Singlet oxygen probe molecules

The molecules available for probing \(^1\)O\(_2\) in natural systems, and specifically for assessing its micro-heterogeneous distribution, are currently not satisfying. Further research is recommended to design a suite of well-behaved, easily accessible and detectable probe molecules. An ideal set of probe molecules should include, those that associate with DOM through electrostatic interaction or by forming a covalent bond, in addition to probe molecules that partition into DOM by hydrophobic interaction. These probe molecules could potentially reveal novel insights into the detailed mechanism of \(^1\)O\(_2\) formation upon irradiation of NOM and the structure of NOM.
The hydrophobic FFA-based probe molecules discussed in chapter 2 did not reproduce the results of a 65-fold higher $^1$O$_2$ concentration in proximity to DOM as detected by TPMA compared to FFA (chapter 3). The challenges of using hydrophobicity to associate the probe molecule with DOM suggest that the pool of hydrophobic pollutants that may be affected by the micro-heterogeneous distribution of $^1$O$_2$ is potentially limited. Thus, enhanced degradation of hydrophobic pollutants due to micro-heterogeneous distribution of $^1$O$_2$ in proximity to DOM may not be as relevant as previously assumed and the partitioning of the pollutant in DOM has to be always verified.

Singlet oxygen production from particulate organic matter

The photochemical properties of POM compared to DOM have been poorly investigated thus far. While the presented study demonstrated $^1$O$_2$ production from POM, it also raised numerous questions regarding the contribution of POM to indirect photochemistry of natural waters. The specific mechanisms of $^1$O$_2$ formation and loss by POM are especially in need of further investigation. The dual probe approach proposed in chapter 3 should be further applied to a variety of organic materials from different sources and of different composition for a systematic assessment of natural POM.

In addition, the possible cause of a lower $^1$O$_2$ production quantum yield of POM compared to DOM needs further assessment. These observations may be an artifact of a higher $^1$O$_2$ quenching rate inside the POM due to reaction with the polymer underlying the organic coating. Therefore, synthetic particles that have the organic matter directly applied on an inert support should be tested.

In addition, the investigation of POM could be extended to other PPRI such as HO• and $^3$DOM. To investigate the extent of the micro-heterogeneous distribution of
other PPRI a dual probe approach for those species should also be considered in accordance to the $^1\text{O}_2$ approach.

It has been recently shown that there is a direct correlation between fluorescence of NOM and $^1\text{O}_2$ production.\textsuperscript{1} Thus, an investigation of the specific optical properties of POM is recommended, by a detailed fluorescence study.

*Quenching kinetics for DOM size assessments*

The approach of using HO• quenching kinetics to estimate the size of DOM led to novel insights into HO• reactivity and structural properties of DOM. The proposed model led to a far-reaching hypothesis that the reactivity of HO• with DOM is dependent on the structure and specific DOM moieties, such as phenols and aromatic compounds. Further research is required to investigate a possible correlation between the specific moieties of DOM and the HO• quenching rate constant. The aromaticity and phenol content of DOM can be easily assessed by excitation-emission matrix.\textsuperscript{2,3} One appealing approach to rapidly and precisely assess the size of DOM may be a multi-parameter model that allows demonstrating moiety-specific HO• quenching rate constants.
5.3 References


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The work described in this thesis could not have been done without the contributions and support of many people.

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